A SEARCH FOR MICROORGANISMS THAT OXIDIZE *CIS*-DICHLOROETHENE AND VINYL CHLORIDE UNDER IRON- OR MANGANESE-REDUCING CONDITIONS

A Thesis

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ABSTRACT

Lesser chlorinated ethenes, *cis*-dichloroethene (cDCE) and vinyl chloride (VC) are reductive dechlorination products of tetrachloroethene and trichloroethene which are among the most-abundant groundwater contaminants in the United States. Due to their ubiquitous and potentially carcinogenic nature, cDCE and VC have been classified as priority pollutants by the United States Environmental Protection Agency.

Through two decades of field study and laboratory research, chlorinated ethenes are found subject to a range of microbial degradation processes, including reductive dechlorination, aerobic oxidation, aerobic cometabolism and anaerobic oxidation. Among these biodegradation mechanisms, anaerobic oxidation of cDCE and VC is the most recently recognized — neither anaerobic chloroethene oxidizing microorganisms nor the pathways involved in these processes have been identified yet. Therefore, acquiring enrichment cultures capable of anaerobically oxidizing cDCE and VC and identifying the mediating organisms is prerequisite to eventual development of molecular biological tools to evaluate the *in situ* presence of such microorganisms.

Microcosm studies on anaerobic oxidation of cDCE and VC under Fe(III)- and Mn(IV)-reducing conditions were conducted in this research. In all, 21 series of microcosms (approximately 350 in total) were prepared, covering 17 sampling locations from 9 chloroethene-contaminated sites or sources throughout the United States, including those where cDCE or VC mineralization under metal-reducing conditions was supposedly observed by previous investigators, or where Fe(III)-reduction is the predominant terminal electron-accepting process. Microcosms were constructed using a variety of site materials, such as aquifer sediments, streambed sediments, soil, groundwater, sludge supernatant, and column contents constructed with tree mulch, cotton gin trash, and river sand. A small amount of yeast extract, along with excess Fe(III) or Mn(IV), was used to rapidly establish metal-reducing

conditions, after which the yeast extract would be depleted and cDCE or VC thereafter would serve as the main electron donors. Change of chloroethenes and accumulation of Fe(II) or Mn(II) in the microcosms were monitored periodically via headspace-sampling/gas-chromatography and a wet-chemistry assay, respectively.

Through a one-and-one-half-year experimental period, anaerobic oxidation of cDCE or VC was not detected in any of the 350 microcosms. No loss of cDCE or VC was detected in 8 series of microcosms, while reductive dechlorination was observed to various degrees in other series, three of which showed complete reductive dechlorination to ethene followed by further reduction of ethene to ethane. Most microcosms that exhibited reductive dechlorination were those unamended with Fe(III) or Mn(IV), but robust reductive dechlorination was also observed in Fe(III)-amended microcosms constructed with organic-rich materials from two sites.

The failure to observe anaerobic cDCE or VC oxidation gave rise to a hypothesis that the ostensibly anaerobic cDCE or VC oxidation observed at some sites or in some laboratory studies might, in fact, have been aerobic oxidation carried out under very low fluxes of oxygen. This hypothesis was based on the low oxygen thresholds previously reported for isolated aerobic VC-oxidizers and on the occurrence of oxygen-contamination reported by other research teams attempting to investigate anaerobic mineralization of cDCE and VC. Therefore, we conducted a subsequent aerobic experiment in which oxygen was added to 16 previously anaerobic microcosms to find out if aerobic cDCE or VC oxidation would occur readily in microcosms that had previously been incubated anaerobically for an extended period. After about 40 days of aerobic incubation, three aerobic VC-oxidizing mixed cultures were obtained.

Future study is thus warranted to investigate whether aerobic VC mineralization can occur in simulated mixed-culture subsurface environments that are

subject to steady influx of oxygen but maintained at extremely low oxygen concentrations.

BIOGRAPHICAL SKETCH

"The flowers and plants withered, the birds' souls disappeared, and the fish passed away. The spring comes again but lacks the beautiful appearance of a hundred flowers, of birds' song, of joyous leaping fish. There is only silence covering a piece of land, forest, and swamp." – <*Silent Spring*>

Disturbed by the reckless use of synthetic chemical pesticides after World War II, Rachel Carson, a famous marine biologist, reluctantly changed her focus in order to warn the public about the long-term effects of misusing pesticides. Her legacy for the beauty and integrity of life continues to inspire new generations, including me, to protect the living world and all its creatures. While reading a good book could change one's life, <*Silent Spring*> first raised my attention to environmental protection, and made me determined to pursue it as a lifelong career.

Jan 5th, 1985, I was born in Qingdao, a most beautiful coastal city in China.

I got my bachelor's degree in water supply and sewage engineering at Qingdao Technological University. Fall 2007, I came to Cornell to pursue a master's degree in Environmental Engineering. Besides the top education at Cornell, I feel lucky to be supervised under Prof. James Gossett, who's so accomplished in chloroethene bioremediation. In addition to professional knowledge and research skills, the most valuable lesson I have learned from Prof. Gossett is the correct attitude of a researcher – committed, modest and persistent. I'm going to start a new academic life at Texas A&M University in the PhD program in Environmental Engineering, Fall 2009. My ultimate goal is to become a good professor in China. Just like Prof. Gossett, I will make encouragement one of the most important factors in my teaching philosophy.

To my family

Jingshuang

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LIST OF ABBREVIATIONS

AFB	Air Force Base
AQDS	anthraquinone-2,6-disulfonate
BES	2-bromoethanesulfonic acid
cDCE	*cis*-1,2-dichloroethene
DCE	dichloroethene
DDI water	distilled-deionized water
DoD	Department of Defense
DS water	distilled water
EISB	enhanced in situ bioremediation
FID	flame ionization detector
GC	gas chromatograph
MBTs	molecular biological tools
MCL	maximum contaminated level
MEK	methyl ethyl ketone
MIBK	methyl isobutyl ketone
MNA	monitored natural attenuation
NAS	Naval Air Station
NAVFAC	Naval Facilities Engineering Command
NPL	National Priorities List
PCE	tetrachloroethene
PRB	permeable reactive barrier

PVC	polyvinyl chloride
RD	reductive dechlorination
TCA	tetrachloroethane
TCD	thermal conductivity detector
TCE	trichloroethene
tDCE	*trans*-1,2-dichloroethene
TEAP	terminal electron-accepting processes
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
VC	vinyl chloride
YE	yeast extract

PREFACE

Drops fall
in the sweet sweeping rush
of the rain
into the sea
into
the cup of its shining waters.
Or
down
to a tranquil,
brown-eyed little river
or the brook
that plunged
over the edge of the cliff
and froze to a stalactite of crystal
it
became
my waterfall.

Donald Culross Peattie and Noel Peattie

<A Cup of Sky>

CHAPTER 1

INTRODUCTION

1.1 Context

Chlorinated solvents, such as tetrachloroethene (PCE), trichloroethene (TCE), *cis*-1,2-dichloroethene (*c*DCE) and vinyl chloride (VC) are among the most frequently detected groundwater contaminants in the United States. More than 1000 sites on 200 military installations were estimated by the U.S. Department of Defense (DoD) to contain chlorinated solvents, resulting from the prevalent use of PCE and TCE as dry cleaning solvents and metal degreasers and the improper methods for their disposal [34]. Remediation of such chlorinated ethene contaminated sites is of great concern due to their toxic and xenobiotic nature. VC is a known carcinogen, while cDCE as well as PCE and TCE are suspected carcinogens [2, 5]. Bioremediation is being heralded as a promising groundwater cleanup technology for chloroethene contaminated sites, especially the development of monitored natural attenuation (MNA) and enhanced in situ bioremediation (EISB) [70, 71, 80].

The occurrence of the less-chlorinated ethenes in the groundwater is due largely to the reductive dechlorination of PCE and TCE, whereby the chlorine substituents are sequentially replaced by hydrogen to yield cDCE and VC. Under anaerobic conditions, the rate of reductive dechlorination declines as the number of chlorine atoms in the chloroethenes decreases [86, 87]. Consequently, although VC can be further degraded to innocuous ethene via complete reductive dechlorination, this process happens at a slow rate often requiring highly reducing methanogenic conditions, which is responsible to a great degree for the accumulation of VC in the subsurface environment [26, 28, 37]. In contrast, as a relatively reduced compound,

VC has a well- documented susceptibility to microbial oxidation processes [41, 42, 86]. Considering that adding oxygen to otherwise anaerobic groundwater aquifers is technically difficult and expensive, anaerobic oxidation of cDCE and VC – if it occurs – provides a potential mechanism for the complete biodegradation of chloroethenes in the groundwater systems.

Through two decades of research, both field evidence and laboratory studies have suggested the possibility of anaerobic oxidation of cDCE and VC. On the one hand, field studies of a number of formerly chloroethene contaminated sites have shown clear evidence of natural attenuation but with an inconclusive mass balance of chlorinated ethenes [22, 91]. These observations indicated that cDCE and VC may have been mineralized to carbon dioxide and chloride through Fe (III) reduction [22, 91]. On the other hand, subsequent laboratory investigations have reported anaerobic oxidation of cDCE and VC under various electron-accepting conditions, such as Fe (III)-, Mn (IV)-, SO_4^{2-} reducing, and methanogenic conditions [9, 10, 12, 13, 14, 85]. In particular, the Fe(III)- or Mn(IV)-linked microbial oxidation could be very significant in the groundwater aquifers, where the oxygen availability is limited.

Unfortunately, anaerobic oxidation of cDCE and VC is still an area under study. No molecular techniques are currently available to confidently and reliably assess the metal-linked oxidative chloroethene degradation at field sites, as the responsible microorganisms involved in these anaerobic oxidations of cDCE and VC still remain unknown, and the underlying mechanisms have not been delineated. For this reason, research is required to better understand these key factors and their overall relevance to the environment. Enhanced knowledge of the metal-linked anaerobic oxidation of cDCE and VC would allow assessment of — and provide increased confidence in — the contributions of these chloroethene degradation mechanisms to MNA and EISB remedies. Reliable MNA or EISB technologies would be expected to

result in increased efficacy and significant cost savings for the remediation of chloroethene contaminated sites versus existing alternatives.

1.2 Objectives

The first objective of this project was to develop enrichment cultures and isolate microorganisms capable of anaerobic oxidation of cDCE and VC under Fe(III)- or Mn(IV)-reducing conditions. Once isolated, the mediating organisms then would be used as model systems to i) elucidate the cDCE and VC anaerobic oxidation pathways, ii) guide the development of molecular biological tools (MBTs) to detect cDCE and VC anaerobic oxidizing bacteria in field samples, iii) develop $^{13}C/^{12}C$ isotopic fractionation signatures of cDCE and VC anaerobic oxidation in field samples, and iv) assess the ubiquity and distribution of cDCE and VC anaerobic oxidizers at field sites. If no organisms capable of anaerobic oxidation of cDCE and/or VC could be isolated from the materials of the candidate sites, the succeeding steps would, of course, not be implemented.

CHAPTER 2

BACKGROUND

2.1 Cis-1,2- Dichloroethene and Vinyl Chloride

2.1.1 Identity, Uses, and Health Effects

1,2-Dichloroethene (1,2-DCE) has been widely detected in groundwater, surface water, industrial effluents, and drinking water supplies throughout the United States. It is a highly flammable and extremely corrosive colorless liquid with a sharp, harsh odor, used as a solvent for waxes, resins, polymers, perfumes, dyes and lacquers [1, 2]. Of the two isomers, *cis*-1, 2-DCE (cDCE) and *trans*-1, 2-DCE (tDCE), cDCE is the more frequently encountered groundwater contaminant, as an intermediate product of anaerobic reductive dechlorination of PCE and TCE present in groundwater [2, 32]. Of 146 groundwater sites on the National Priorities List (NPL), which contains the most serious hazardous waste sites in the nation identified by the U.S. Environmental Protection Agency (USEPA), 130 have been detected with cDCE. The maximum contaminated level (MCL) for cDCE in drinking water is 0.07 mg/L [2].

While lower oral doses of cDCE have effects on the blood, such as decreased numbers of red blood cells, and effects on the liver, extremely high doses of cDCE orally administered to animals may cause death. Lethal symptoms in animals exposed orally to cDCE involve central nervous system depression and respiratory depression. The USEPA has placed cDCE in a non-cancer or a 'not classifiable' category 'D' [2].

At room temperature, vinyl chloride (VC) is a colorless gas with mildly sweet odor, which burns easily and is not stable at high temperatures [4, 5]. Most of the VC produced in the United States (approximately 98%) is used for the industrial manufacture of polyvinyl chloride (PVC), which has been widely used in manufacture

of pipes, furniture, packaging products, automotive parts, wire and cable coatings, and a variety of other products [4, 5].

VC is a known carcinogen, as determined by the U.S. Department of Health and Human Services. As demonstrated in numerous studies of workers in the VC production industry, chronic exposure to VC can result in hepatic angiosarcoma, a very rare liver cancer in humans. Other results of exposure to VC on human health include narcotic effects, Raynaud's phenomenon (problems with the blood flow in the hands – fingers turn white and hurt when they go into the cold), scleroderma-like skin changes, and hepatocellular alterations [4, 5]. In addition, breathing extremely high levels of VC is lethal. The MCL for VC in drinking water is 0.002 mg/L [4, 5].

2.1.2 Environmental Fate and Transport

While massive synthesis of various industrial solvents and PVC plastics can generate contaminant effluents containing DCE and VC respectively, incomplete reductive dehalogenation of PCE and TCE is likely the most prevalent source of these reduced daughter products in subsurface environments. The primary transport process for VC in most surface waters is rapid volatilization to the atmosphere, on the order of hours or days. However, groundwater systems are gas-phase limited and unmixed: therefore volatilization is limited. Biotransformation of cDCE and VC can occur through a variety of mechanisms, including: i) anaerobic reductive dechlorination to ethene and/or ethane [27, 37]; ii) anaerobic oxidation to CO_2 under humic acid conditions [11] or CO_2 and CH_4 under methanogenic conditions [14, 85]; iii) anaerobic oxidation to CO_2 using a number of inorganic electron acceptors, such as Fe (III), Mn (IV), and $SO4^{2-}$ [9, 10, 12, 13]; iv) aerobic cometabolic oxidation to CO_2, using methane, propane, methanol, ammonia as the co-substrates [33]; v) aerobic

oxidation to CO_2 utilizing cDCE or VC as primary growth substrate [24, 25]; and vi) reduction through a range of metal and metal-oxide surface catalyzed reactions [20].

2.2 Biodegradation of DCE and VC

2.2.1 Reductive Dechlorination of Chloroethenes

Microbial reductive dechlorination is the best understood chloroethene degradation pathway under anaerobic conditions, wherein chloroethenes serve as electron acceptors and require the supply of molecular hydrogen [29, 39, 44, 67, 81] or in some instances acetate, as the electron donors[18, 44]. Because of the electron-acceptor role served by chloroethenes, the process is sometimes called "chlororespiration" [50, 67, 77]. Complete reductive dechlorination involves the sequential dechlorination of PCE to TCE to DCEs to VC to ethene (Figure 2.1), which can be further reduced to ethane under highly reducing conditions, e.g., methanogenesis [27]. The combined production of ethene and ethane, therefore, is commonly applied to evaluate the extent of reductive dechlorination of chloroethenes, especially VC, at field sites [18, 90]. The efficiency of reductive dechlorination is greatly varied *in situ,* and a full dechlorination to ethene or ethane is difficult to achieve due to numerous factors, such as the redox characteristics of the chlorinated compounds, the presence of competent dechlorinators, and the supply of electron donors and the like [18, 51].

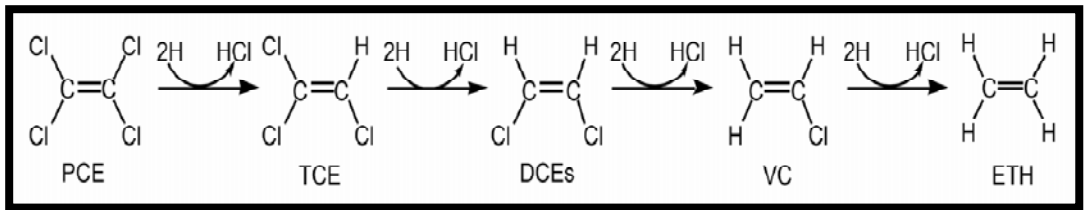


Figure 2.1 Complete reductive dechlorination process from PCE to ethene [67].

The rate and extent of reductive dechlorination are directly proportional to the number of chlorine substituents attached to the carbon atoms. As highly oxidized compounds, PCE and TCE readily undergo a reduction process, whereas the dechlorination of DCE and VC traditionally occur at relatively slow rates, especially from VC to ethene, which is considered the rate-limiting process in complete reductive dechlorination [26, 67].

The results of many previous studies have indicated that complete reductive dechlorination of chloroethenes may be ascribed to the cooperative activities of multiple microbial consortia instead of one single species [26]. While a fairly large number of pure cultures capable of reductive dechlorination of PCE to TCE or to DCE have been developed, several bacterial species coupling growth to efficient degradation of DCE and VC but not PCE have also been isolated, including *Dehalococcoides* sp strain BAV1 and *Dehalococcoides* sp strain FL2 [26, 68, 47]. Up to now, the only identified organism that catalyzes full reductive dechlorination from PCE to ethene is *D. ethenogenes* Strain 195 [26, 36, 48, 67]. However, the final dechlorinating step, VC to ethene, has been proved to be rather slow and apparently a cometabolic transformation in Strain 195 [26, 67, 82].

The supply of electron donor is another major factor affecting the efficiency of reductive dechlorination. Since groundwater aquifers are usually oligotrophic, electron donors can often become the limiting factor for the full dechlorination of chloroethene contaminants [51]. Molecular hydrogen is the electron donor directly used by nearly all bacteria that carry out reductive dechlorination of chloroethenes; however, under *in situ* conditions, H_2 may serve as electron donor for multiple indigenous organisms mediating other reductive processes, resulting in the competition between the reductive dechlorinators and other hydrogentrophs. The minimum H_2 requirement, or H_2 threshold, required by organisms mediating different metabolic processes is in the

following order (high to low): acetogenesis > methanogenesis > sulfate-reduction > reductive dechlorination > iron-reduction > manganese-reduction > nitrate-reduction [54, 66, 94] (Table 2.1). With a H_2 threshold value well below that of homoacetogens and hydrogenotrophic methanogens, reductive dechlorinators should commonly outcompete these two classes of hydrogen consumers under electron-donor limiting conditions [18]. And while it is commonly suggested that the biotransformation of VC to ethene or ethane demands highly reducing methanogenic conditions, examination of the hydrogen thresholds makes it clear that it is possible for complete reductive dechlorination to happen in the mildly reducing environments predominated by SO_4^{2-} reduction — or even in systems characterized as Fe(III)- or Mn(IV)-reducing, when

Table 2.1 H_2 threshold and $\Delta G^{0'}$ values for different redox conditions.

Process	**H_2 threshold [nM]**	**$\Delta G^{0'}$ [kJ/mol H_2]**
Acetogenesis	336-3,640	-26.1
Methanogenesis	5-95	-33.9
Sulphate reduction [$SO_4^{2-} \rightarrow HS^-$]	1-15	-38
Reductive dechlorination	<0.3	-130 to -187
Iron reduction	0.1-0.8	-228.3
Manganese reduction	<0.05	—[a]
Nitrate reduction [$NO_3^- \rightarrow N_2O$, N_2]	<0.05	-240

Data from Maurice L.G. C. Luijten et al. 2004[66] and Löffler et al. 1999 [54].
a. *Value is indicated in neither reference.*

sufficient electron donors are provided [54, 94]. Complete dechlorination of cDCE and VC to ethene or ethane in the presence of Fe(III) or Mn(IV) was observed in our microcosm studies (as is later presented in this thesis).

In summary, anaerobic reductive dechlorination is the most significant biotransformation pathway for the natural attenuation of more-chlorinated ethenes, PCE and TCE. However, incomplete reductive dechlorination is frequently observed

at field sites due to one or more factors (e.g., inadequate dechlorinating populations; insufficient electron donor; competing microbial processes; unfavorable environmental conditions), resulting in the accumulation of cDCE and VC — the latter being of special concern because it is considered more toxic than its more-chlorinated precursor compounds. Therefore, it has been proposed that the combination of reductive dechlorination with microbial oxidation under either aerobic or anaerobic conditions can be a successful strategy for the in situ bioremediation of chloroethenes at some sites.

2.2.2 Aerobic Oxidation of cDCE and VC

Aerobic oxidations of cDCE and VC are cometabolic processes in some microorganisms, but there are bacteria capable of growth-coupled aerobic oxidation of cDCE or VC in which they are used as primary substrates and carbon sources.

Aerobic cometabolic oxidation of chlorinated solvents requires the existence of oxygen as well as an additional carbon/energy source to achieve the normal metabolic functions of the responsible organisms [69]. These organisms decompose the chloroethene compounds more or less fortuitously as the result of certain non-specific oxygenases but derive no energy-conserving or growth-linked benefit from the oxidation process [18, 69]. Effective oxygenase inducing substrates for VC or cDCE cometabolism include methane, and the end products of reductive dechlorination, ethene and ethane [75, 82, 83, 84]. Additionally, cDCE can also be cometabolized by VC oxidizers and aromatic compound oxidizers [18, 84], while VC cometabolism can also use a range of other substrates such as propane, butane, propene, isoprene, and ammonium [82]. However, as a remediation technology, cometabolic oxidation requires the administration of suitable cosubstrate (and probably oxygen, too) to the subsurface — a challenging requirement. As for non-engineered remediation (MNA),

cometabolism of cDCE or VC is likely to be limited to interfacial "fringe" regions where anaerobic plumes (in which cDCE and/or VC are produced via reductive dechlorination, along with potential cosubstrates such as methane) meet aerobic conditions [18].

Significant mineralization of cDCE under aerobic conditions without the addition of co-substrates has been observed in microcosm and enrichment culture studies [24]. 14±2% and 67±11% of [1, 2-^{14}C]DCE was recovered as $^{14}CO_2$ in microcosms inoculated with aquifer and creek-bed sediments, respectively, from a former drum disposal site at Naval Air Station, Cecil Field. However, none of the responsible microorganisms were identified in these studies. To date, the only organism isolated that can utilize cDCE as the sole carbon and energy source is *Polaromona vacuolata* strain JS666 [24]. JS666 may be a prime candidate for bioaugmentation at sites where cDCE has migrated into aerobic zones, in that growth-linked oxidation of cDCE appears to be uncommon at field sites, and JS666 can obtain rapid cDCE removal rates without the need for co-substrates.

Another study by Coleman *et al.* revealed that aerobic bacteria capable of the growth-coupled oxidation of VC are likely to play a major role in the natural attenuation of VC due to their wide distribution and kinetic characteristics, and consequently are equally as important, if not more important, than organisms carrying out cometabolic VC oxidation [25]. Twelve different strains of bacteria were isolated from 23 out of 37 microcosms and enrichments inoculated with material from 22 different sites in the USA, Germany and Australia: 11 of them are *Mycobacterium* strains and one is a *Nocardioides* strain. Other bacteria that are capable of growth on VC as a sole carbon and energy source, including the first characterized strain *Mycobacterium* L1 and *Pseudomonas aeruginosa* strains MF1 and DL1, had been previously isolated by Hartmans and deBont, and Verce *et al.,* respectively. VC

oxidation metabolism of these organisms is likely to contain an initial step catalyzed by an inducible alkene monooxygenase to generate the VC epoxide, chlorooxirane [42] (Figure 2.2).

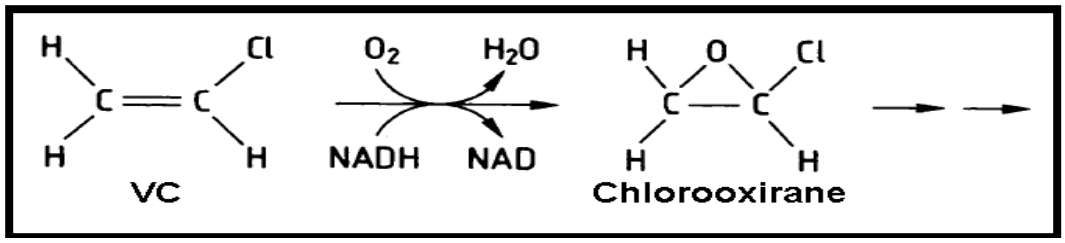


Figure 2.2 Initial step of aerobic oxidation of VC catalyzed by alkene monooxygenase [42].

Furthermore, it is worth noting that five of 12 strains of bacteria isolated by Coleman *et al.* were further studied with respect to their oxygen-utilizing kinetics. All were found to effectively biodegrade VC under extremely low oxygen concentrations indicated by the low half-velocity constant (Ks) measured (from 0.03 to 0.3 mg/liter). The minimum oxygen requirement (i.e., threshold) needed to carry out the VC oxidation metabolism by these microorganisms was as low as 0.02 to 0.1 mg/L (Table 2.2). These results, along with the field observation that VC often disappears in the aerobic zones downgradient of chloroethene contaminated sites, suggests that VC-assimilating bacteria are potentially responsible for the successful in situ bioremediation of VC.

Table 2.2 Oxygen half-velocity constants of VC-assimilating bacteria [25].

Strain	$K_s(O_2)$ [mg/liter]	O_2 threshold [mg/liter]
Mycobacterium		
JS60	0.17 ± 0.06	0.02 ± 0.01
JS61	0.03 ± 0.04	0.07 ± 0.01
JS616	0.3 ± 0.24	0.10 ± 0.02
JS617	0.07 ± 0.06	0.06 ± 0.02
Nocardioides sp. strain JS614	0.11 ± 0.04	0.06 ± 0.01

2.2.3 Anaerobic Oxidation of cDCE and VC

Field studies of many originally chloroethene-contaminated sites have shown lines of evidence of natural attenuation, but with an indecisive mass balance of the chlorinated compounds. A former fire training area at Plattsburgh Air Force Base, New York, has a history of chloroethene contamination including TCE, DCE and VC, in the groundwater systems. Early studies at this site illustrated that VC, the reductive dechlorination product of TCE and DCE, accumulated near the fire-training pit. However VC was further degraded downgradient with a lack of ethene/ethane accumulation, most likely via Fe(III) reduction or aerobic respiration [91].

A similar situation related to the biotransformation of chlorinated ethenes occurred at Naval Air Station (NAS) Cecil Field, Florida [22]. A methanogenic zone was present in the groundwater near the contaminant source, which gave preference to the reductive dechlorination of PCE, TCE and DCE, and was surrounded by an iron-reducing zone further downgradient, where anaerobic oxidation of VC to CO_2 appeared favorable. These postulated processes are highly consistent with the observed behavior of chloroethenes at this site. PCE, cDCE and VC were present in ground water near the contaminant source but dropped below detectable levels along the flowpath. In the downgradient zone, without measurable chloroethenes, there were elevated concentrations of dissolved inorganic carbon and chloride. These observations suggested that chloroethenes probably had been transformed to CO_2 and chloride through the combined effects of reductive dechlorination in the methanogenic zone and oxidation in the downgradient, iron-reducing and aerobic zones.

Besides circumstantial field evidence, laboratory investigations have suggested a number of mechanisms for anaerobic oxidation of VC to carbon dioxide and/or reduction to methane (Figure 2.3). The potential for anaerobic oxidation of chloroethenes was first reported by Vogel and McCarty in 1985 in a small continuous-

flow fixed-film methanogenic column fed with [^{14}C]PCE [85]. In addition to TCE, DCE and VC, up to 24% radiolabeled PCE was recovered as $^{14}CO_2$ in the effluent. The addition of unlabeled VC to the column feed caused a decrease of $^{14}CO_2$ activity to 12%, whereas the cessation of VC supply increased the $^{14}CO_2$ activity approximately back to the original level (23%), which led to the hypothesis that $^{14}CO_2$ production is a result of [^{14}C]VC mineralization. Furthermore, this study suggested a potential pathway for microbial oxidation of VC, which may involve a hydration reaction to yield chloroethanol followed by the oxidation to aldehydes and ultimate mineralization to CO_2 or CO_2 and CH_4. However, since none of these hypothetical intermediates was detected in the study, this pathway is best described as speculative.

Studies by Bradley and Chapelle suggested the potentially important role of oxidative acetogenesis in anaerobic mineralization of VC to CO_2 or CO_2 and CH_4 [15, 16]. In their research of anaerobic degradation of [1,2-^{14}C]VC under methanogenic conditions, equimolar recovery of $^{14}CO_2$ and $^{14}CH_4$ (22±2% and 22±1% respectively) was observed without delay [14]. This observation gave rise to the hypothesis that VC mineralization involves an initial fermentation step to yield acetate, because acetate is the only known two-carbon-compound substrate for methanogenesis that typically accounts for 70% of CH_4 production in the methanogenic process, and more importantly, it distinctively generates CO_2 and CH_4 in equal amounts [15, 16, 31]:

$$CH_2CHCl(g) + 2H_2O(l) \rightarrow CH_3COO^-(aq) + H_2(aq) + 2H^+(aq) + Cl^-(aq)$$

$$\Delta G^{0'} = -62.4 kJ / mol$$

$$CH_3COO^-(aq) + H^+(aq) \rightarrow CH_4(aq) + CO_2(aq)$$

$$\Delta G^{0'} = -51.1 kJ / mol$$

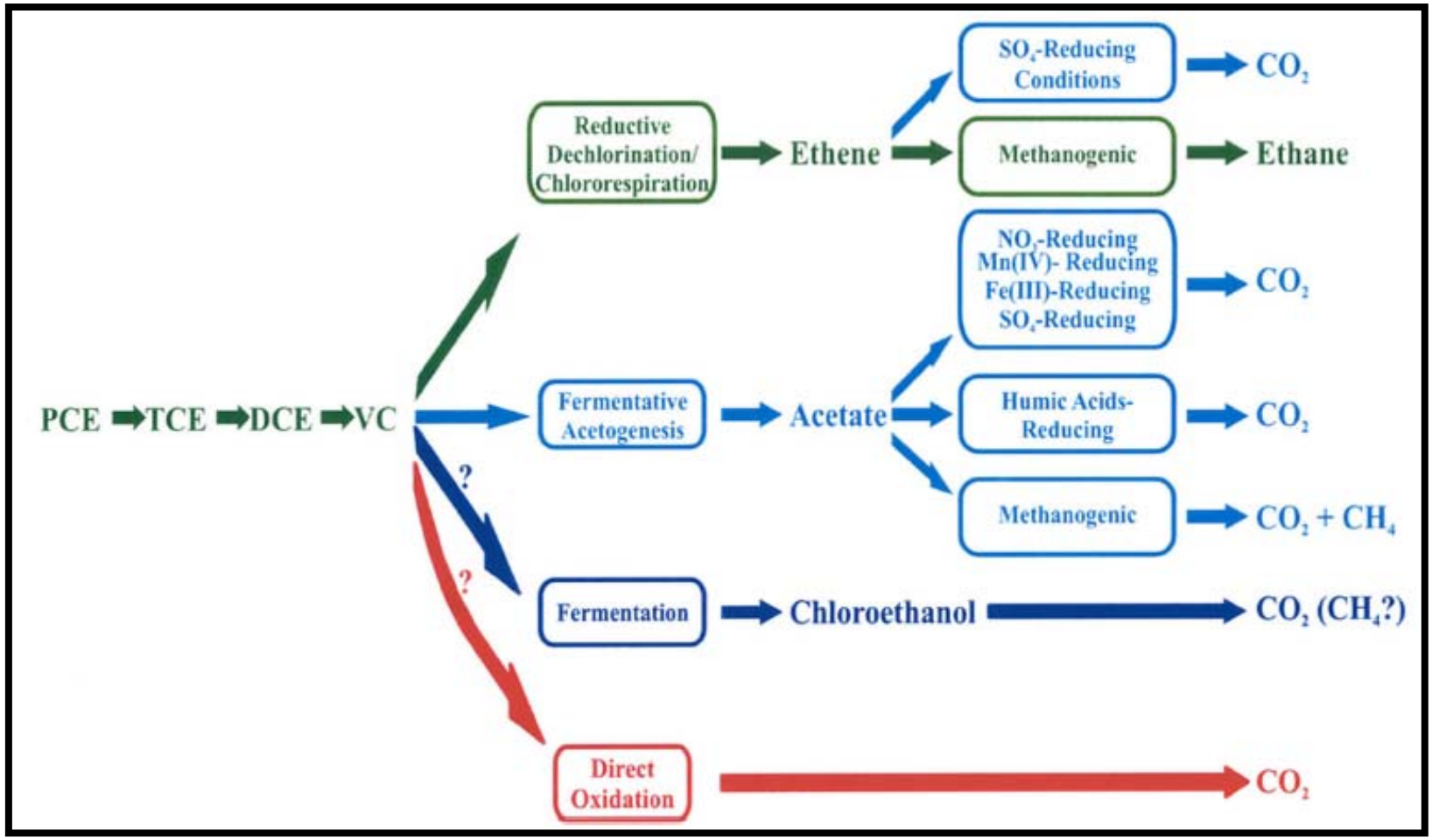


Figure 2.3 Complete anaerobic biodegradation pathways of chloroethenes via reductive dechlorination and microbial oxidation [18].

To further corroborate this assumption, microcosms were set up using carboxyl-labeled acetate ([1-^{14}C]acetate) and methyl labeled acetate ([2-^{14}C]acetate). 75±2% [1-^{14}C]acetate was recovered as $^{14}CO_2$ within 24 days, and a total amount of 74±7% [2-^{14}C]acetate was recovered as $^{14}CO_2$ and $^{14}CH_4$ with 55±7% and 19±5% of the initial radioactivity respectively. Subsequent investigation confirmed the immediate and transient conversion of [1,2-^{14}C]VC to [^{14}C]acetate was associated with concomitantly rapid increase of $^{14}CO_2$ and $^{14}CH_4$ [16]. The immediate conversion of [^{14}C]acetate to $^{14}CO_2$ and $^{14}CH_4$ observed in these studies was consequently supportive of the hypothesis that acetotrophic methanogenesis was directly involved in the VC mineralization [15].

Bradley *et al*. also tested the contribution of methanogens to the degradation of [1,2-^{14}C]VC to $^{14}CO_2$ and $^{14}CH_4$ by adding 20mM 2-bromoethanesulfonic acid (BES, a known inhibitor for methanogenesis) [15]. In the BES-unamended microcosms, 14±2% [^{14}C]VC was recovered as $^{14}CH_4$ and 63±3% as $^{14}CO_2$. In contrast, while $^{14}CH_4$ production was completely inhibited in the microcosms with BES, an almost equal amount decrease (13±2%, compared with 14±2%) of $^{14}CO_2$ recovery(51±2%) was observed. The amount of $^{14}CO_2$ recovery was approximately 36% greater than that of $^{14}CH_4$. These results implied that, i) methanogens were responsible for the biodegradation of VC to CH_4; ii) and apart from methanogenesis, an undefined nonmethanogenic mechanisms may be predominantly accountable for VC mineralization to CO_2. Since O_2, NO_3^-, Fe(III), and SO_4^{2-} reduction were calculated to be insignificant in the cultures, previously identified humic acid reduction became a suggested mechanism for the anaerobic oxidation of VC observed in the study.

Microcosm studies initiated by the same research team previously demonstrated that humic acid, a ubiquitous constituent of soil and sediments, is capable of serving as the electron acceptor for the efficient mineralization of both VC

and DCE [63, 76]. The streambed sediment sample used in this study was characterized to contain rich organic matter and saturated with humic acid — the same material used in the oxidative acetogenesis studies. In the microcosms unamended with humic acid, an immediate 39±3% of original radioactivity of [^{14}C]VC was recovered as $^{14}CO_2$ in 50days, and $^{14}CO_2$ was the only observed product without the accumulation of $^{14}CH_4$. The amount of [^{14}C]VC recovered (39±3%) was comparable to that observed in the oxidative acetogenesis study — a 36% access of $^{14}CO_2$ recovery with respect to $^{14}CH_4$ when methanogenesis was not suppressed, indicating that humic acid reduction might be the nonmethanogenic metabolism hypothesized responsible for the VC mineralization observed in that study. Compared with VC mineralization under humic acid-reducing conditions, the recovery of [1,2-^{14}C]DCE was relatively low. While only 7±1% [1,2-^{14}C]DCE recovered as $^{14}CO_2$ and trace amounts of VC, ethene and ethane were detected in the unamended microcosms, the addition of humic acid substantially stimulated [1,2-^{14}C]DCE recovery to 25±6% as $^{14}CO_2$, and no VC, ethene or ethane were observed.

Due to the lack of detectable intermediates, the acetate formed during oxidative acetogenesis is also suggested to be capable of being utilized by various inorganic acceptors, such as Fe(III), Mn(IV) and SO_4^{2-}, in anaerobic mineralization of VC. Bradley and Chapelle provided the first evidence [9] and a number of reports of anaerobic VC oxidation under Fe(III)-reducing conditions [9, 10, 12, 13]. Experiments were conducted using shallow aquifer sediment collected from one site of NAS Cecil Field, with the absence of detectable chlorinated contaminants, and two sites at Plattsburgh Air Force Base, within an anaerobic chloroethene plume. All microcosms from the three sites showed fast mineralization of [1,2-^{14}C]VC to $^{14}CO_2$ within 84 hours. Following experiments with microcosms from NAS Cecil Field further affirmed that the evaluated amount of $^{14}CO_2$ recovery was consistent with the[^{14}C] VC

loss. The excess Fe(II) accumulation (380±100 nmol observed *vs*. 82±34 nm expected) was also measured, implying that VC mineralization might be coupled to Fe(III) reduction.

Finally, another mechanism contributing to the anoxic oxidation of VC may involve an initial reductive dechlorination of VC to ethene followed by mineralization of ethene to CO_2 via SO_4^{2-} reduction [17, 18]. In experimental treatments amended with SO_4^{2-}, 84±12% of [1,2-^{14}C]ethene was recovered as $^{14}CO_2$ accompanied by simultaneous SO_4 reduction (>40%) and dissolved sulfide (880±90 μM) production (92±3% of [1,2-^{14}C]ethene retained in the killed control). In contrast, in microcosms unamended with SO_4^{2-}, which proved to contain active methanogens, stoichiometric reduction of ethene to ethane was observed. In light of these observations, ethene was suggested as an alternative intermediate, besides acetate, formed during net microbial oxidation of VC to CO_2. However, in a previous study that used shallow aquifer sediments from another site, [1,2-^{14}C]ethene was not detected during the mineralization of [1,2-^{14}C]VC to $^{14}CO_2$ under Fe(III)-reducing conditions [9]. It is possible, therefore, that ethene as an intermediate for anaerobic VC oxidation may not be widespread.

In a nutshell, the characterized mechanisms of anaerobic VC oxidation involve an initial conversion to acetate, which can be further fermented to CO_2 and CH_4 via acetotrophic methanogens; or oxidized to CO_2 via humic acid reduction or under a range of inorganic electron accepting conditions, such as Fe(III)-, Mn(IV)-, SO_4^{2-}- and NO_3^--reductions. Alternatively, ethene could also be an important intermediate product for anoxic VC mineralization to CO_2. Finally, when an appropriate terminal electron acceptor is available, VC might be directly oxidized to CO_2 without the accumulation of any intermediate products [9, 18]. However, further investigations are needed to

validate these hypotheses. Potential mechanisms for anaerobic microbial oxidation of VC and DCE are summarized in Table 2.3.

Table 2.3 Anaerobic oxidation of DCE and VC summary.

	Electron Acceptors					Reference
	Fe(III)	Mn(IV)	SO_4^{2-}	CH_4	Humic Acid	
VC	✓					9, 10, 12
			✓			12, 17
				✓		12, 14, 15, 85
					✓	11
DCE	✓					10, 12
		✓				13
			✓			12
				✓		12
					✓	11

The effect of redox conditions on anaerobic DCE and VC mineralization has been investigated in microcosm studies [12]. Compared with VC oxidation, rate and extent of DCE oxidation was much lower under each electron-accepting condition examined; and anaerobic DCE mineralization was half as fast as aerobic DCE mineralization. Mineralization of [1, 2-^{14}C]VC decreased under increasingly reducing conditions, in the order of aerobic> Fe (III)-reducing> SO_4^{2-}-reducing> methanogenic conditions. On the other hand, the rate and magnitude of [1, 2-^{14}C]DCE oxidation did not differ significantly between iron-reducing, SO_4^{2-}-reducing and methanogenic conditions. Combined with the fact that [^{14}C]VC, [^{14}C]ethene, and [^{14}C]ethane were also detected in the headspace of DCE microcosms, it was proposed that the anaerobic oxidation of DCE may involve an initial, rate-limiting reduction step and Fe(III) and SO_4^{2-} may not be adequately powerful oxidants to improve DCE oxidation[10, 12]. Subsequently, Mn(IV), as a stronger oxidant than Fe(III) and SO_4^{2-}, was demonstrated

to successfully oxidize [1,2-^{14}C]DCE to $^{14}CO_2$ with the absence of detectable reductive dechlorination products[13].

Kinetic work on anaerobic mineralization of DCE and VC in creek bed sediments under methanogenic and Fe(III)-reducing conditions has been reported [10]. VC mineralization exhibited Michaelis-Menten kinetics for VC concentrations ranging from 0.2-57 μM, with a maximum oxidation rate of 0.76±0.07 μM d^{-1} under Fe(III)-reducing conditions, which was four times greater than that under methanogenic conditions (0.19±0.01 μM d^{-1}). The metabolism involved in VC mineralization via Fe(III) reduction had a greater affinity for VC than that via methanogenesis, indicated by a lower K_m value (1.3±0.5 μM and 7.6±1.7 μM under Fe(III)-reducing and methanogenic conditions, respectively). The anaerobic oxidation of DCE, on the other hand, could be described by first-order kinetics under examined DCE concentrations (1.4-80 μM)—the rate of DCE mineralization did not show considerable difference between Fe(III) reduction and methanogenesis (both about 0.6±0.2% d^{-1}). The results on the kinetics of anaerobic mineralization of DCE and VC provided further support for the conclusion that the mechanisms underlying these two processes are dramatically distinct from each other [10, 12, 18].

Through two decades of field and laboratory research, knowledge on the degradation pathways of anaerobic mineralization of VC and DCE has been greatly improved. Consensus has been reached that the combined effort of reductive dechlorination followed by anaerobic oxidation can provide a propitious alternative for the complete biodegradation of chlorinated ethenes. However, further scientific research is required to develop a pure culture that is capable of anaerobic oxidation of the lesser chlorinated ethenes and clearly identify the underlying mechanisms, or the application of *in situ* bioremediation can not be a successful remedy for chloroethene

contaminated sites. The metal-linked oxidative mechanism of DCE and VC through Fe(III) and Mn(IV) reduction is the point of interest in this research.

2.3 Fe(III) and Mn(IV) Reduction

Iron and manganese, as the two most abundant redox-active metals in the Earth's crust, have shown great potential to serve as the electron acceptors in the mineralization of diverse organic contaminants in a wide range of subsurface environments [59, 64, 72]. Under anoxic conditions, toluene, xylene, phenol, benzene, benzoate, benzylalcohol, benzaldehyde, and other recalcitrant organic contaminants have been reported to be extensively oxidized to CO_2 by the Fe(III)- and/or Mn(IV)-respiring microorganisms coupled to energy conservation [57]. Furthermore, anaerobic microbial oxidation of cDCE and VC under Fe(III)- and/or Mn(IV)-reducing conditions has been observed by Bradley and Chapelle [9, 10, 12, 13].

The abundance of Fe(III) and its availability to readily convert between ferric (Fe(III)) and ferrous (Fe(II)) states have made Fe(III) reduction an important process for the oxidation of organic matter to CO_2 in sedimentary environments [64, 72]. A higher concentration of Fe(III) is frequently found in aquatic sediments than that of other electron acceptors such as oxygen, sulfate and nitrate [57]. Although Mn(IV) oxides are typically only 10% as plentiful as Fe(III) oxides, a larger proportion of Mn(IV) oxides is expected to be available for microbial reductions and Mn(IV) reduction is more energetically favorable [13, 61]. In most conditions, Mn(II) is the end product of Mn(IV) reduction, sometimes with Mn(III) as an intermediate.

Apart from the rich contents of Fe(III) in groundwater sediments, it is interesting to note that the Fe(III)-reducing zone often appears immediately downgradient of the contaminant plume, due to the zone's ability to migrate over time [64]. Concomitant with the development of anaerobic conditions and the relocation of

the Fe(III)-reducing zone further downgradient, another electron-accepting process, normally methanogenesis, will appear near the contaminant source taking the place of Fe(III) reduction. In the case of chloroethene degradation, this distribution of electron-accepting zones promotes the complete biotransformation of chlorinated ethenes in two aspects. On the one side, it favors the reductive dechlorination of the parent compounds (i.e., PCE and TCE) to the lesser-chlorinated ethenes (i.e. cDCE and VC) under methanogenic conditions close to the contaminant source; on the other side, it supports the anoxic oxidation of the daughter products to CO_2 under Fe(III)-reducing conditions. Thus, the movement of the Fe(III)-reducing zone gives more weight to the importance of Fe(III) reduction in subsurface environments. The distribution of predominant redox conditions along the flow path in deep pristine aquifers and shallow contaminant aquifers is shown in Figure 2.4.

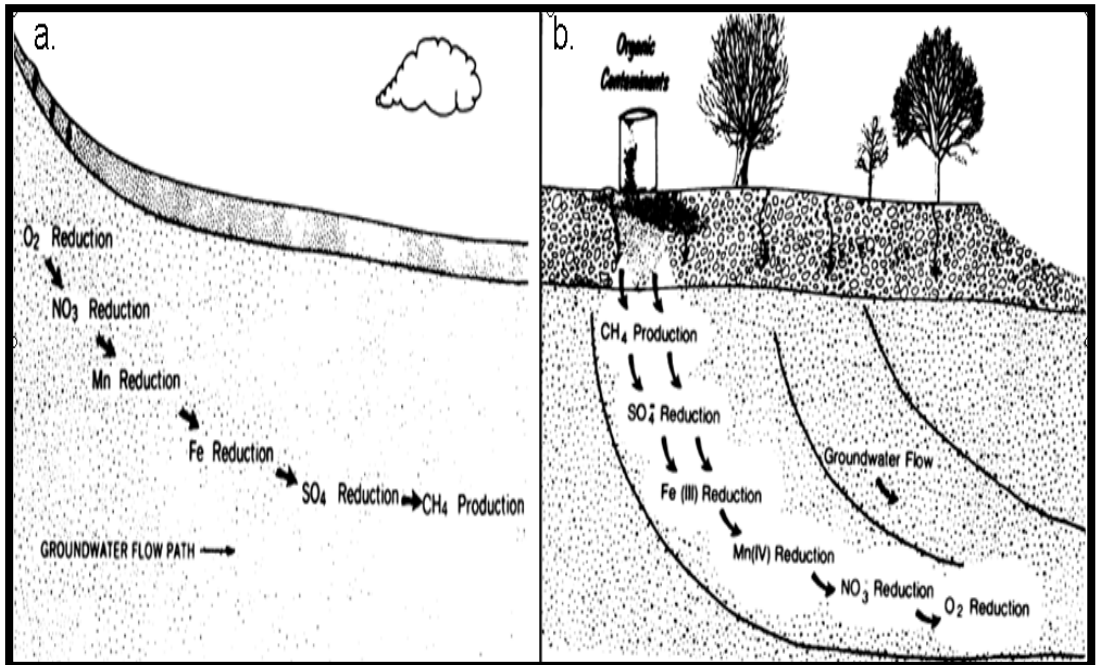


Figure 2.4 Typical distribution of electron-accepting process in aquatic sediments of deep pristine aquifers (a) and shallow aquifers contaminated with organic compounds (b) [61].

The form of Fe(III) and Mn(IV) oxides also plays a major part in the decomposition of the organic matters associated with Fe(III) and Mn(IV) reduction [61, 62, 72]. Specifically, it determines the rate and extent of the Fe(III)- and Mn(IV)-respiring process. A number of studies have proved that ill-defined crystalline Fe(III) oxides perform higher Fe(III) reduction rates than the more crystalline Fe(III) oxides [9, 57]. For example, in the enrichment cultures inoculated with the tidal river and estuarine sediments, Lovley *et al.* demonstrated that glucose was metabolized by amorphous Fe(III) oxyhydroxide 10-fold faster than hematite (essentially Fe_2O_3). Furthermore, the fermentable substrates, such as acetate, butyrate and hydrogen were completely oxidized with the simultaneous increase of Fe(II) when amorphous Fe(III) oxyhydroxide served as the electron acceptor, whereas only small quantities of Fe(II) accumulated when hematite was the potential electron acceptor [57]. The form of Mn(IV) is also believed to influence the rate and extent of Mn(IV) reduction, but it has not been comprehensively studied [61].

Briefly, Fe(III) reduction has long been suggested among the earliest significant forms of microbial respiration, which is still playing an important role in the modern sedimentary environment. Owing to the abundance of Fe(III) and Mn(IV) in the anaerobic sediments, Fe(III) and Mn(IV) respirations have the potential to be major mechanisms for the mineralization of various organic contaminants in groundwater systems when their oxides are in a suitable form. Fe(III) and Mn(IV) reductions may play a significant role in the remediation of chloroethene contaminated sites.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Strategy

The purpose of the experiments described herein was to develop enrichment cultures capable of anaerobic oxidation of cDCE and VC under Fe(III)- or Mn(IV)-reducing conditions that will further promote the determination of the actual pathway(s) of cDCE and VC anaerobic oxidation and the development of MBTs for field validation of these processes. Anaerobic microcosms were prepared using materials (e.g., sediment, soil, groundwater, and wastewater sludge) obtained from nine candidate sources for anaerobic oxidation, including sites where mineralization of cDCE and/or VC under metal-reducing conditions was allegedly observed by previous investigators, or sources which exhibited metal-reducing (primarily Fe(III)-reducing) conditions. Some of the materials were available only in sediment form, or in small quantity, in which cases an enrichment medium was prepared based on a modified recipe of Lovley and Philip's for Fe-reducing systems [57]. To quickly establish a metal-reducing environment, freshly precipitated Fe(III) or Mn(IV) was added to the microcosms, along with the addition of a small amount of yeast extract. Both Fe(III) and Mn(IV) were in excess over the yeast extract added. Thus, the yeast extract could create metal-reducing conditions rapidly, after which the yeast extract would be depleted by Fe- or Mn- reducers and cDCE or VC thereafter would serve as the main substrates.

Remaining masses of cDCE/VC and the accumulation of Fe(II)/Mn(II) in the microcosms were monitored periodically (and typically over at least 6 months) via headspace-sampling/gas-chromatography and a wet-chemistry assay, respectively. If

cDCE or VC levels decreased with accumulation of Fe(II) or Mn(II) in the same microcosms, the presence of microorganisms capable of anaerobic oxidation of cDCE/VC would be further confirmed. Unfortunately, however, since none of the microcosms exhibited anaerobic oxidation of cDCE or VC within the experimental period, subsequent characterization could not be performed.

After the failure to identify microorganisms capable of anaerobic cDCE/VC oxidation, a hypothesis was raised that what has appeared to be anaerobic oxidation of cDCE and VC at some sites or in some laboratory studies might actually have been aerobic oxidation sustained under extremely low fluxes of O_2. This hypothesis results from two sources: 1) the low oxygen thresholds reported for known, aerobic oxidizers of VC [25]; and 2) personal communications we received from two other research teams (Paul Bradley & Frank Chapelle; and David Freedman & Stephen Zinder) who had observed VC-depletion in ostensibly anaerobic microcosms and reactors, but who had also discovered that staff had unintentionally allowed small amounts of air to enter the systems during sampling events. Freedman & Zinder even isolated a strain of VC-oxidizing *Mycobacterium* from their supposed anaerobic microcosms. We therefore thought it entirely possible that at least some reports of "anaerobic oxidation" could have, in fact, been the result of aerobic processes. To begin exploring this possibility, microcosms that had been previously operated anaerobically were now provided with oxygen to ascertain whether or not cDCE/VC degradation might commence aerobically after extended anaerobic incubation. Headspace samples were analyzed for cDCE/VC and O_2 via gas chromatography.

3.2 Chemicals, Stock Solutions, and Medium

cis-1,2-Dichloroethene (≥99%; Tokyo Chemical Industry Co. Ltd.), and gaseous vinyl chloride (≥99.5%; Sigma-Aldrich Co. Ltd.), were used as culture

substrates as well as analytical standards. A saturated cDCE stock solution was prepared by adding 1.5 mL neat cDCE to a 160 mL serum vial full of DS water and sealed with Teflon®-lined, butyl-rubber septum and aluminum crimp cap(solubility of cDCE is approximately 7 g/L; the density of cDCE is 1.28 g/mL). Ultra high purity compressed ethene obtained from Airgas and ethane with ≥99% purity from Sigma-Aldrich were used as analytical standards.

Amorphous Fe(III) oxyhydroxide suspension was prepared following the method of Lovley and Phillips [57]. A $FeCl_3$ (98%; Aldrich Chemical Co. Ltd.) stock solution of 400 mM was neutralized with NaOH (97%; FisherChemical) solution to reach pH 7. The resultant Fe(III) oxyhydroxide suspension was triply centrifuged at 5,000 rpm for 10 min and rinsed with distilled water so as to remove Cl^- ions. The Fe(III) stock suspension achieved a final concentration of 200 mM Fe(III). Amorphous Mn(IV) suspension (as MnO_2) was made by oxidizing 12 mM $MnCl_2$ (Mallinckrodt Ltd.) with 8 mM $KMnO_4$ (Mallinckrodt Ltd.) in a solution of NaOH (0.8794 g was added) [73]. The resulting suspension was subsequently centrifuged at 5,000 rpm for 10 min and washed with DS water. This centrifugation/washing procedure was repeated twice to remove chloride ion. The final concentration of Mn(IV) suspension was about 180 mM Mn(IV). Fe(III) and Mn(IV) stock suspensions were kept in 160 mL serum bottles and purged with N_2 for 30 min. The bottles were then sealed with Teflon®-lined, butyl-rubber septa, crimped with aluminum crimp caps, and stored at 4℃ until use.

The enrichment medium was composed of the following ingredients: 2.5 g/L $NaHCO_3$, 0.1 g/L $CaCl_2{\cdot}2H_2O$, 0.1 g/L KCl, 1.5 g/L NH_4Cl, 0.69 g/L $NaH_2PO_4 \cdot 2H_2O$, 0.1 g/L NaCl, 0.1 g/L $MgCl_2{\cdot}6H_2O$, 0.01 g/L $MgSO_4{\cdot}7H_2O$, 0.005 g/L $MnCl_2{\cdot}4H_2O$, 0.001 g/L $Na_2MoO_4{\cdot}2H_2O$, and 0.01-0.05 g/L yeast extract. The medium was purged with N_2/CO_2 (80/20) gas for 30 min before use.

An O_2 scrubbing solution was used to absorb trace amounts of O_2 from the purge-gas mixture, which was composed of 10 mL/L 20% titanous chloride solution (FisherScientific), 4.412 g/L sodium citrate dehydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$; FisherChemical), and 12.5 g/L sodium bicarbonate (NaH_2CO_3; Mallinckrodt).

3.3 Experimental Setup

3.3.1 The Addition of Electron Donors and Electron Acceptors

The nominal concentration (i.e., ignoring partitioning to headspace) of chlorinated ethene substrates added to microcosms was 10 mg/L, which is much greater than typical cDCE/VC contamination levels in a chloroethene plume. Three concentrations of yeast extract (YE), 0.01 g/L, 0.025 g/L and 0.05 g/L were administered to microcosms (of 100-mL liquid volume) for different sources according to the characteristics of the materials we obtained.

$$10\ mg/L\ cDCE = \frac{10\ mg/L}{97\ g/mol} = 103\ \mu M\ cDCE = 10.3\ \mu mol/bottle\ cDCE$$

$$10\ mg/L\ VC = \frac{10\ mg/L}{62.5\ g/mol} = 160\ \mu M\ VC = 16\ \mu mol/bottle\ VC$$

The μmoles of Fe(III) and Mn(IV) required for the stoichiometric oxidation of cDCE, VC and yeast extract are listed in Table 3.1.

Table 3.1 Calculation of electron acceptors required per bottle.

Electron Donor (ED)	ED amount (μmol)	μeeq EA required	Fe(III) required (μmol)	Mn(IV) required (μmol)
cDCE	10.3	82.4	82.4	41.2
VC	16	160	160	80

Based on the calculation above, 5 mL of 200 mM Fe(III) oxyhydroxide stock solution and 5 mL of 180 mM Mn(IV) stock suspension were added to microcosms

designated as receiving Fe or Mn, respectively, resulting in added levels of 1000 μmol Fe(III) or 900 μmol Mn(IV) — excesses in all cases. Another evidence for the excessive dosage of electron acceptors in the YE-amended microcosms was that the formation of Fe(II) or Mn(II) during the incubation period was much lower than the initial amount of Fe(III) or Mn(IV) added – based on data presented later in "Results".

3.3.2 Preparation of Microcosms

Microcosm studies were initiated using site materials that were obtained from nine candidate sources in the USA. These are (in chronological order): (1) contents from two different columns, simulating a permeable reactive barrier (PRB) constructed with magnetite and bark mulch at Altus Air Force Base, OK; (2) supernatant from the anaerobic sludge digester of the Ithaca Area Wastewater Treatment Plant, Ithaca NY; (3) groundwaters from three locations influenced by a chloroethene plume from a former fire training pit (FT-02) at Plattsburgh Air Force Base, NY: (4) sediments from Naval Submarine Base Kings Bay Outcrop and Kings Bay KBA-11-13A, GA; (5) sediment from Naval Air Station, Jacksonville, FL; (6) soil and adjacent ground waters from three undisclosed Superfund sites, CA; (7) old microcosms from Cardinal Landfill, NH, that had been operated under anoxic conditions; (8) sediments from two locations at Aberdeen Proving Ground, MD; and (9) streambed sediment and nearby groundwater from Naval Air Station Cecil Field, FL, the location being one where Bradley and Chapelle observed anaerobic oxidation of VC and cDCE many years ago [10, 12, 14].

Series of microcosms (21 series in all) were prepared, each of which usually consisted of eight "treatments" (including four treatments that were amended with Fe(III)), with each treatment prepared in duplicate. However, two series (one for Ithaca Area Wastewater Treatment Plant and one for Cecil Field), which additionally

employed Mn(IV) as electron acceptor, had four additional treatments. More Fe-reducing microcosms were constructed than Mn-reducing microcosms due to the fact that most site materials were from sources where iron reduction was a prevailing process. Types of microcosms are shown in Table 3.2.

Table 3.2 Types of microcosms.

cDCE-fed	VC-fed
live w/ Fe	live w/ Fe
live w/Mn	live w/Mn
live w/o Fe/Mn	live w/o Fe/Mn
autoclaved controls w/ Fe	autoclaved controls w/ Fe
autoclaved controls w/Mn	autoclaved controls w/Mn
autoclaved controls w/o Fe/Mn	autoclaved controls w/o Fe/Mn

Microcosms were created by transferring inoculum site material to each serum bottle followed by the dispensing of the enrichment medium, and Fe(III) oxyhydroxide stock slurry (for Fe(III)-reducing treatments), or Mn(IV) stock slurry (for Mn(IV)-reducing treatments) or anoxic DS water (for unamended treatments), to make a final volume of approximately 100 mL. Microcosms were kept purging with a mixed gas of N_2/CO_2 during the whole process of experimental setup. The volume ratio of N_2:CO_2 was adjusted to achieve a neutral pH (6.8-7.2) in the microcosms. They were then sealed with Teflon® coated butyl-rubber septa and aluminum caps. Killed controls were autoclaved for 45 min at 130℃. cDCE and VC were added to designated bottles at the final nominal concentration of 103 μM and 160 μM, using a volume of about 150 μL (saturated aqueous stock) or 40 μL (gas), respectively. Finally, the prepared microcosms were inverted, and agitated on an orbital shaker for 1 hr at 120 rpm, then incubated (inverted) quiescently in the dark at 22℃. Microcosms prepared with sludge supernatant were incubated at 30 ℃.

Slight differences occurred in the amounts of inoculum material used and the concentration of yeast extract in the medium, depending on the different characteristics of the site materials we received. The summary tables of microcosm preparation are shown in Appendices I and II.

3.3.2.1 Site/Source 1: PRB Columns from Altus Air Force Base, Site SS-17 Oklahoma

Two column materials were provided by John Wilson (USEPA Kerr Lab, Ada, OK) from the effluent ends of two continuous-flow columns that were constructed to model the permeable reactive barrier (PRB or biowall) at the Altus AFB SS-17 site, Oklahoma. Mulch column B2 was packed with a mixture of plant mulch and river sand, which originally had a significant amount of biologically available Fe(III). Column B4 had mulch and hematite and limestone added to buffer pH. In a recent column study by Shen and Wilson [78], it was discovered that the TCE supplied to these column influents was effectively removed, only 1% of which was accounted for as cDCE, VC, ethene, ethane and acetylene; however, up to 56% of [1, 2-^{13}C]TCE added after 353 days of operation was recovered as $^{13}CO_2$ and no greater than 0.6% of ^{13}C-TCE was recovered as $^{13}CH_4$ [79]. Meanwhile, significant sulfate reduction and iron sulfide accumulation was observed in all columns with plant mulch, but a greater rate constant associated with FeS was achieved in the column amended with mulch and hematite [78]. Although the authors attributed the efficient removal of TCE to abiotic degradation, iron-reducing bacteria could have played a major role in the oxidation process due to the fact that the materials used to construct the columns had high concentrations of iron, 1800±130 mg/kg.

To investigate the potential contribution of biological processes to anaerobic mineralization of the chloroethenes, we obtained 30 mL inoculum materials from each column — B2 and B4. Column B2 was filled with 50% (v/v) shredded tree mulch, 10%

(v/v) cotton gin trash, and 40% (v/v) river sand; Column B4 was constructed with the same material, except for the addition of 32% (v/v) sand, 4% (v/v) granular hematite, and 4% (v/v) crushed limestone. The chemical composition of the plant mulch is listed below in Table 3.3.

Two series of microcosms were set up using inoculum materials from the effluent ends of columns B2 and B4. Each microcosm contained 1.5 mL column material from the respective columns, 94mL medium with 0.05 g/L yeast extract, and 5mL Fe(III) stock slurry, or 5 mL anoxic distilled water (DS water) in the Fe(III)-amended or unamended microcosms, to make a final volume of approximately 100 mL.

Table 3.3 Major component of the tree mulch and cotton gin [79].

Component	Percentage (%) in the Plant Biomass on a weight basis (Mean ± Standard deviation, n=3)	
	Tree Mulch	Cotton Gin Trash
Cellulose	37.1 ± 2.05	39.6 ± 1.63
Hemicellulose	19.4 ± 0.85	19.9 ± 2.71
Lignin	4.7 ± 1.93	9.6 ± 0.23
Total Ash	28.48±1.88	14.2 ± 0.25
Total Nitrogen	0.44 ± 0.02	1.3 ± 0.02
Total Carbon	34.9 ± 2.08	41.1 ± 0.50
Phosphorous	0.032 ± 0.002	0.145 ± 0.006
Aluminum	0.39 ± 0.044	0.13 ± 0.014
Calcium	0.96 ± 0.101	1.34 ± 0.12
Iron	0.32 ± 0.035	0.097 ± 0.012
Potassium	0.33 ± 0.004	1.5 ± 0.045
Magnesium	0.19 ± 0.017	0.32 ± 0.016
Sodium	0.051 ± 0.007	0.09 7± 0.02
Sulfur	Not Detected	0.16 ± 0.021

3.3.2.2 Site/Source 2: Ithaca Area Wastewater Treatment Plant, Ithaca, New York

Given that digester sludge is a microbe-rich material containing a range of organic matter and plenty of other nutrients, we obtained 2 L mixed digester sludge from the anaerobic digester at the Ithaca Area Wastewater Treatment Plant, which has

been receiving chlorinated contaminants for a long period of time. The sludge was settled for several days before using it, to allow separation of the supernatant from the sediment.

Twenty-four microcosms were prepared with 10 mL sludge supernatant as inoculum material and 85 mL medium containing 0.05 mg/L yeast extract. Some also received 5 mL of Fe(III) sock slurry or 5 mL of DS water. In addition to investigating the effects of Fe(III) amendment, we also prepared microcosms with 5 mL of 180-mM Mn(IV) stock slurry.

3.3.2.3 Site/Source 3: Plattsburgh Air Force Base, Plattsburgh, New York

Groundwater samples were provided by Dave Farnsworth (BRAC Envir. Coord., Plattsburgh), from three locations at Plattsburgh Air Force Base (fire training area FT-002) in New York. Two liters of groundwater was sampled from the influent to the Groundwater Operable Unit, a groundwater treatment system. The dissolved oxygen (D.O.) and the redox potential (Eh) for the groundwater sample were 3.89 mg/L and -471 mV, respectively. Water samples were collected from the Idaho Avenue Discharge Pipe and East Flightline Discharge Pipe – both receiving groundwaters from subsurface interceptor trenches. D.O. and Eh for the samples from the former location were 2.91 mg/L and -350 mV; and 2.68 mg/L and -572 mV for the latter location.

Three series of microcosms were set up using the sampled groundwaters as the inocula. Each microcosm contained: 85 mL medium with yeast extract concentration of 0.025 g/L; 10 mL water sample from the corresponding locations; 5 mL 200 mM Fe(III) stock slurry or 5 mL anoxic DS water in the Fe(III)- amended or -unamended microcosms.

3.3.2.4 Site/Source 4: Naval Submarine Base, Kings Bay, Georgia

Fe-reducing sediment material was provided by Paul Bradley (USGS) from two locations at Naval Submarine Base, Kings Bay. Two series of microcosms were prepared using sediment from Kings Bay Outcrop, sampled at an exposed outcrop of shallow aquifer; and Kings Bay KBA-11-13A, collected from the top organic layer in a shallow aquifer system which receives substantial oxygenated infiltration. In the previous assay conducted by Bradley *et al.*, these materials had shown mineralization activity with [1, 2-^{14}C]VC/DCE at dissolved oxygen concentration below 25 μg/L (results not published).

Microcosms for Kings Bay KBA-11-13A had 9 g sediment as inoculum; microcosms for Kings Bay Outcrop were amended with 5 mL shallow aquifer sediment. Both series of microcosms contained 95 mL medium, with a yeast extract concentration of 0.01 g/L.

3.3.2.5 Site/Source 5: Naval Air Station, Jacksonville, Florida

Site material from Naval Air Station (NAS) Jacksonville was provided by Paul Bradley (USGS). It consisted of freshwater aquifer sediment material.

One series of 16 microcosms was prepared from this material. Each microcosm contained 5 mL freshwater sediment, 90 mL medium with 0.01 g/L yeast exact, and either 5 mL 200 mM Fe(III) stock slurry or 5 mL anoxic DS water in the unamended microcosms.

3.3.2.6 Site/Source 6: Unidentified Superfund Sites, Southern California

Site materials were provided by David Freedman of Clemson University from three undisclosed Superfund sites in Southern California. These were sites used to investigate the anaerobic oxidation of VC several years ago and one had yielded an enrichment in which VC mineralization was observed without addition of electron acceptors (Note that the enrichment activity was later lost) [49]. Groundwater and soil

samples were available from these three sites though not all species of sample from each. Three series of microcosms were prepared using site materials from the three different locations. For Series #1, we received groundwater from a single well (MW 2116) and two samples of soil from MW-2113-48 – #2151 and #2119. For Series #2, groundwater and soil was received from an unspecified monitoring well. For Series #3-(a) and -(b), site materials consisted of groundwater from one well and soil from two different cores.

Microcosms for Series #1 contained 90 mL groundwater sample and 5 g soil collected from each of the two cores. The inoculum for Series #2 consisted of 7 g soil sample and 90 mL mixture of groundwater and medium in a ratio of 10:8. Series #3-(a) contained 90 mL groundwater sample and 5 g soil from each of the two cores; microcosms for Series #3-(b) were composed of 90 mL mixture of 95% groundwater and 5% medium, along with 5 g soil from each core. The mixture of groundwater and medium for this series was refrigerated overnight before preparation of microcosms. Because of concerns that this groundwater-medium mixture used to prepare Series #3-(b) had been exposed to low levels of O_2, microcosms were prepared again for this series in the same manner — designated as Series #3-(c). The concentration of yeast extract in the enrichment medium was 0.01 g/L for all microcosms, and 5 mL Fe(III) stock slurry was amended to the Fe(III)-reducing microcosms, while 5 mL anoxic DS water was added to the unamended microcosms.

3.3.2.7 Site/Source 7: Cardinal Landfill, Farmington, New Hampshire

The Cardinal Landfill site has been contaminated by cleaning solvents (PCE, TCE, methylene chloride) and paint solvents (acetone, ketone, xylenes, toluene) since the 1960s [3]. However, according to field data over the years, cDCE and VC have been declining along the groundwater flow path — and the decline is not accounted for by ethene. Furthermore, the dissolved iron levels at this site are in excess of 100

mg/L throughout [Evan Cox (Geosyntec), personal communication]. We obtained three microcosms that had been prepared from site materials by U. Toronto researchers under the direction of Elizabeth Edwards; they were provided to us by Melanie Duhamel; they had been amended with sulfate and were labeled SO_4-1A, SO_4-2A, and SO_4-3A. The particular site location from which these microcosms were constructed contains a mixture of cDCE, dichloroethanes, acetone, ketones, methyl ethyl ketone (MEK), and methyl isobutyl ketone (MIBK). Each microcosm contained 30 mL sediment and 180 mL groundwater collected from well SW111-Deep and had been amended with sulfate. The addition of sulfate was demonstrated by another lab to promote the anaerobic oxidation of ketones. However, the degradation of cDCE under Fe(III)-reducing conditions was not tested previously with these materials. Thus, we prepared new microcosms from these earlier, sulfate-amended microcosms to stimulate Fe(III)-reducing activity which might be tied to the potential anaerobic oxidation that appears to be occurring in the field at Cardinal Landfill.

Two series of microcosms were created for the Cardinal site. The contents of the first series included: i) 90 mL mixture of the supernatants from microcosms SO4-1A and SO4-2A (7%, v/v), groundwater from Superfund series #3 (5%, v/v), and medium with 0.01 g/L yeast extract (88%, v/v); ii) 5 g soil from Superfund subset #3; iii) 5 mL 200mM Fe(III) stock suspension or 5 mL anoxic DS water in the Fe(III)-amended or -unamended microcosms. (Note that materials from Superfund Series #3 were used in these Cardinal-site microcosms due to a misunderstanding with my advisor when we discussed the proposal for experimental setup.) Microcosms for the second treatment consisted of 90 mL mixture of 3% (v/v) supernatants from microcosm SO4-3A and 97% (v/v) medium (yeast extract concentration 0.01 g/L), plus 5 mL Fe(III) suspension or anoxic DS water for the Fe(III)-amended or -unamended microcosms.

3.3.2.8 Site/Source 8: Aberdeen Proving Ground, Maryland

Freshwater tidal wetland sediments were provided by E. Majcher (Geosyntec, Inc) from two locations, WB23 and WB30, along West Branch Canal Creek, Aberdeen Proving Ground, Maryland. This study site receives the discharge from a contaminated groundwater plume containing tetrachloroethene (TCE) and tetrachloroethane (TCA). Field and laboratory evidence have shown that DCE and VC accumulated during the biotransformation of TCE and TCA were completely degraded, but the dechlorination product ethene was rarely detected [55, 56]. The degradation of VC was found to be coupled with an increased proportion of *Methanosarcinaceae*, a group that generally encompasses acetotrophic methanogens. It was therefore suggested that the presence of iron in the wetland porewater may have stimulated the growth of acetate-utilizing methanogens, which might in turn promote the mineralization activity of the lesser chlorinated ethenes at this site.

Two series of microcosms were prepared using sediment materials sampled from sites WB23 and WB30, respectively. Microcosms contained 25 g sediment from the corresponding site, 77.5 mL medium (yeast exact concentration=0.01 g/L), 5 mL 200 mM Fe(III) stock slurry or 5 mL anoxic DS water in the Fe(III)-amended or -unamended bottles.

3.3.2.9 Site/Source 9: Naval Air Station, Cecil Field, Florida

Rowell Creek, Site 3 at Cecil Field is where Bradley and Chapelle obtained most of their field samples from which they observed anaerobic oxidation of DCE and VC under various electron accepting conditions, primarily associated with iron reduction, but also manganese reduction, sulfate reduction, humic acid reduction, as well as methanogenesis. Streambed sediments and groundwater from this same location (Rowell Creek, Site 3) were newly sampled and provided by Mike Singletary (NAVFAC). Groundwater was sampled from monitoring well CEF-003-GW-31S

(31S). The sediment samples, which were labeled SD-1, SD-2, SD-3, were collected from three locations from a depth of 2-10 inches below the sediment/water interface. SD-1 was located approximately 9 ft upstream of 31S; SD-2 was located approximately 6 ft upstream of 31S; and SD-3 was located approximately 5 ft downstream of 31S.

Two series of microcosms were prepared for Cecil Field site. Series #1 contained 16 microcosms, and each of them was prepared with 90 mL groundwater sample and 25 g mixed sediment of SD-1, SD-2 and SD-3 (1:1:1); Series #2 involved 16 microcosms, amended with 100 mL mixture of 50% groundwater and 50% medium (yeast extract concentration = 0.01 g/L), and 25 g mixed sediment from SD-1, SD-2 and SD-3 (1:1:1); Series #3 comprised 22 microcosms, which contained 85 mL medium (no yeast extract) and 20 g mixed sediment collected from the three locations (1:1:1). 5 mL 200 mM Fe(III) stock slurry was added to the Fe(III)-reducing microcosms; 5 mL anoxic DS water was added to the unamended microcosms. For Series #3, eight microcosms amended with Mn(IV) as the electron acceptor were prepared. However, due to the lack of medium prepared, only 1 killed control fed with cDCE and VC was created, resulting in a total number of 22 microcosms for Series #3.

3.4 Analytical Methods

3.4.1 Gas Chromatography

cDCE, VC, ethene, ethane, methane and O_2 were monitored by a PerkinElmer AutoSystem Gas Chromatograph (GC) equipped with an 8-feet × 1/8-inch stainless-steel column, packed with 1% SP-1000 on 60/80 Carbopack-B (Supelco Inc). Two methods were employed to measure the volatile organic compounds and O_2. To analyze the chloroethenes, 100 μL headspace samples were injected to the GC column using a gas-tight locking syringe. The sample flow was routed to a flame ionization

detector (FID) at an initial temperature of 90℃ for 2.5 min, and then the temperature ramped at 30 ℃ per minute to 195 ℃ for the next 3.5 min of the run. This method gave retention times for methane, ethene, ethane, VC, cDCE of 0.58 min, 0.75 min, 0.82 min, 2 min, and 5.4 min, respectively. 500 µL headspace samples were injected to analyze O_2. The samples initially flowed through a thermal conductivity detector (TCD) for the measurement of O_2. After 0.76 min, a switching valve routed the sample flow to the FID for the measurement of the volatile organic compounds. The column was kept isothermally at 90 ℃ for 2 min after the injection, and the retention time for O_2 in the method was 0.56 min. Since only O_2 was measured with this method, the run was stopped after O_2 had eluted. N_2 was used as the carrier and reference gas, with a flowrate of 30 mL/min. The flowrates for H_2 and air were 45 mL/min and 450 mL/min, respectively.

3.4.2 Analysis of total Fe, Fe(II) and Mn(II)

3.4.2.1 Total Fe

The total iron in microcosms was analyzed with the FerroZine method (method 8147, Hach Company, Loveland, CO), with a measurement range of 0.009 to 1.400 mg/L Fe. The reagent solution contains ammonium thioglycolate, thioglycolic acid, demineralized water and other components (not listed). This method is based on the paper by Stookey in Analytical Chemistry (vol. 42, 779-781), in which hydroxylamine was used to reduce ferric iron to ferrous iron, and then the trace amount of ferrous iron in the samples could react with ferrozine (3-(2-pyridyl)-5,6-bis-(phenylsulfonic acid)-1,2,4-triazine) to form a stable magenta complex. The purple color is directly proportional to the iron concentration, which can then be determined according to the absorbance reading on a spectrophotometer.

0.1 mL aliquots of microcosm slurry were transferred to 50 mL centrifuge tubes, and diluted to 25 mL with distilled-deionized (DDI) water. The reagent solution in the pillows (Hach Company) was then added to the tubes, and mixed well with the contents. After a 5 min reaction time, the samples were centrifuged (IEC Centra MP4R Centrifuge) at 1000 rpm for 2 min before analyzing the absorbance of the supernatant on a Hewlett Packard 8452A Diode Array Spectrophotometer at 562 nm. A sample blank without reagent and a reagent blank with DDI water were prepared in the same manner. The total iron concentration in the microcosms was calculated by subtracting the iron concentration of the sample blank from that of the samples, as the absorbance reading of the reagent blank was negligible.

3.4.2.2 Fe(II)

The accumulation of Fe(II) was analyzed using the phenanthroline method (method 8146, Hach Company), with a measurement range of 0.02 to 3.00 mg/L Fe(II). The reagent powder for Fe(II) analysis contains 1,10-phenathroline and bicarbonate, which will give an orange color when ferrous iron is present.

A 0.5-mL, well-mixed aliquot was transferred from microcosms to the 50-mL centrifuge tubes. 15 μL of 2 N hydrochloric acid was added to adjust the pH of the sample to about 3.5. And the sample was then diluted to 25 mL with DDI water, mixed well with the reagent powder (Hach Company) and settled for 3 min for full color development. Afterwards, the samples were centrifuged at 1000 rpm for 2 min before reading the absorbance of the supernatant on a spectrophotometer at 510 nm. A sample blank and a reagent blank were prepared as previously described.

However, when Fe(II) measurement was conducted with microcosms that contained muddy and suspended sediments, the results could be highly affected by the rate and time of centrifugation. To improve the accuracy of this method, filtration was employed to measure the accumulation of Fe(II) in the sediment-rich microcosms,

including those from Undisclosed Superfund sites, Cardinal, Aberdeen and Cecil Field sites. The diluted sample, therefore, was first filtered (0.45-μm HV Durapore® Membrane Filter, Millipore) to obtain a clarified solution and then mixed with the reagent. After a 3-min reaction time, the absorbance of the sample was analyzed by the spectrophotometer. Since the samples after filtration was very clear, a sample blank was not used in the filtration method.

3.4.2.3 Total Mn and Mn(II)

The same method was used for the analysis of total Mn and Mn(II) — Method 8034 for Mn measurement from Hach Company, with a range of 0.1 to 20 mg/L. Since this method is used to detect all Mn species, filtration was performed to measure Mn(II) in order to eliminate undissolved Mn species from the filtrate, while no filtration was conducted to measure total Mn.

0.1 mL aliquot of well-mixed sample was transferred to a 50-mL centrifuge tube and diluted to 10 mL with DDS water. The solution was then filtered, followed by the addition of 6 μL 2N hydrochloric acid to adjust the pH to 3-5. After citrate buffer powder and sodium periodate (Hach Company) were added, the sample was shaken to mix well with the reagent and allowed to sit for 5 min for color development. Afterwards, the absorbance of the samples was measured on the spectrophotometer at 525 nm. A sample blank and a reagent blank were prepared for determining the final concentration of Mn(II), as described previously.

3.4.3 Preparation of Standards

Quantification of each compound in the microcosms was determined by comparison of: i) the peak area values given by the data integrator connected to GC (for chloroethenes and other gases); and ii) the absorbance value given by the data-analyzing software connected to the spectrophotometer (for total Fe, Fe(II) and,

Mn(II)) to the predetermined corresponding standard curves. Standards were prepared by adding known amounts of compounds to 160-mL sealed serum vials with 100 mL of DS water (with the exception of O_2), purging the headspace with N_2/CO_2 at 80:20 ratio, and allowed to agitate on the orbital shaker for hours to reach equilibrium before analysis.

3.4.3.1 cDCE Standards

A 10 g/L stock solution of cDCE (≥99%; Tokyo Chemical Industry Co. Ltd.) in methanol was prepared by spiking 78 μL neat cDCE into 10-mL methanol. Standards were then created by delivering 10/25/50/100/200 μL stock solution to sealed serum vials to give 1/2.5/5/10/20 mg/L standards. 0.1-mL headspace samples were quantified with GC, yielding a calibration curve representing the linear relationship between the peak areas and amounts (in μmol/bottle) of cDCE in the bottles. Two cDCE standard curves were prepared, at 22 ℃ and 30 ℃ respectively.

3.4.3.2 VC /Ethene/ Ethane Standards

VC(Sigma-Aldrich)/Ethene/Ethane(Airgas) standards were created by transferring known volumes of gases to the serum vials. The moles of gases delivered were calculated by the ideal gas law: $PV = nRT$, using the temperature and the pressure at the time of preparation. 0.1 mL headspace samples were quantified by GC, yielding a calibration curve representing the linear relationship between the peak areas and amounts (in μmol/bottle) of VC/Ethene/Ethane in the bottles. Two VC standard curves were prepared, at 22 ℃ and 30 ℃, respectively.

3.4.3.3 O_2 Standards

For oxygen — unlike with other analytes — we were primarily interested in knowing the aqueous concentrations in microcosms, rather than total quantities per bottle. Gaseous O_2 standards were prepared by adding known volumes of O_2 to 160 mL N_2 filled serum vials. The moles of O_2 delivered were determined according to the

ideal gas law: $PV = nRT$, using the temperature and the pressure at the time of preparation. The aqueous concentrations of O_2 that would be at equilibrium with these various gaseous concentrations were calculated from Henry's Law: $c = k_h p$, where c is the aqueous O_2 concentration (mol/L); p is the partial pressure of O_2 (atm); k_h is Henry's Law constant, 0.0013 $mol \cdot L^{-1} \cdot atm^{-1}$ for O_2 at 298K. Headspace samples of 0.5 mL were quantified with GC, yielding a calibration curve representing the linear relationship between the peak areas and equilibrium aqueous O_2 concentrations (in mg/L) in bottles.

3.4.3.4 Total Fe/Fe(II)/Total Mn/Mn(II) Standards

A 100-mg/L Total Iron Standard Solution and a 250-mg/L Manganese Voluette Ampule Standard Solution were obtained from Hach Company. A 200-mg/L Fe(II) standard stock solution was prepared by dissolving 0.7 g ferrous ammonium sulfate ($Fe(NH_4)_2(SO4)_2 \cdot 6H_2O$, Hach) in a 500-mL volumetric flask. Standards were then developed by diluting the corresponding standard solution to known concentrations and transferred to the cuvettes to test the absorbance with the spectrophotometer.

CHAPTER 4

RESULTS

4.1 Results for Anaerobic Microcosms

All graphs of chloroethenes, iron and manganese measurements are shown in Appendix III – Results for Anaerobic Microcosms. Plotted data are generally means ± standard deviation for duplicate microcosms (error bars are smaller than the symbols where not visible). In case where duplicates showed marked disagreement, data are presented for both separately. Total iron concentration, which was an average value of that in Fe(III)-amended, live and killed microcosms, was only measured at the beginning when microcosms were constructed, and supposed to remain conservative. Therefore, this line is horizontal in all the graphs (with the exception of microcosms amended with sludge supernatant from the Ithaca Area Wastewater Treatment Plant).

4.1.1 Site/Source 1: PRB Columns from Altus Air Force Base, Site SS-17, Oklahoma

4.1.1.1 Column B2

About 10 μmol cDCE and 16 μmol VC were initially added to the designated microcosms (Figure 4.1). After 158 days of incubation, no loss of cDCE or VC was observed in either the microcosms amended with iron or without iron. The average concentration of total iron in the cDCE and VC bottles were approximately 562 mg/L and 554 mg/L, respectively. Fe(II) increased rapidly in the live bottles during the first 13 days and remained at a level of 200-300 mg/L thereafter until the termination of monitoring. The accumulation of Fe(II) can be attributable to biological activity since Fe(II) concentration remained at the background level in the killed controls. However,

the formation of Fe(II) wasn't associated with the biodegradation of cDCE or VC, which was the interest of this study.

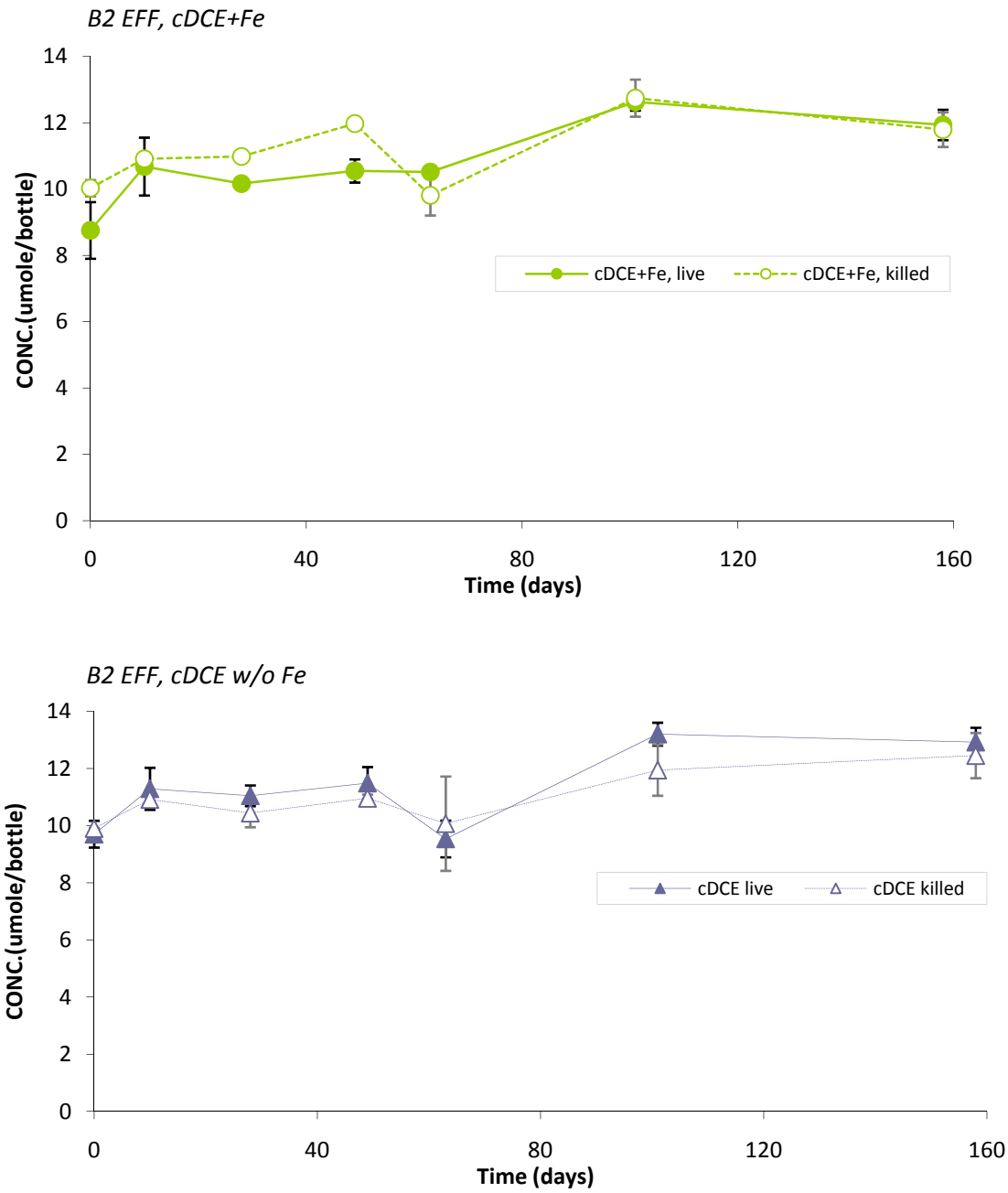


Figure 4.1.a Anaerobic microcosm results for Altus AFB Column B2: cDCE in cDCE-fed bottles.

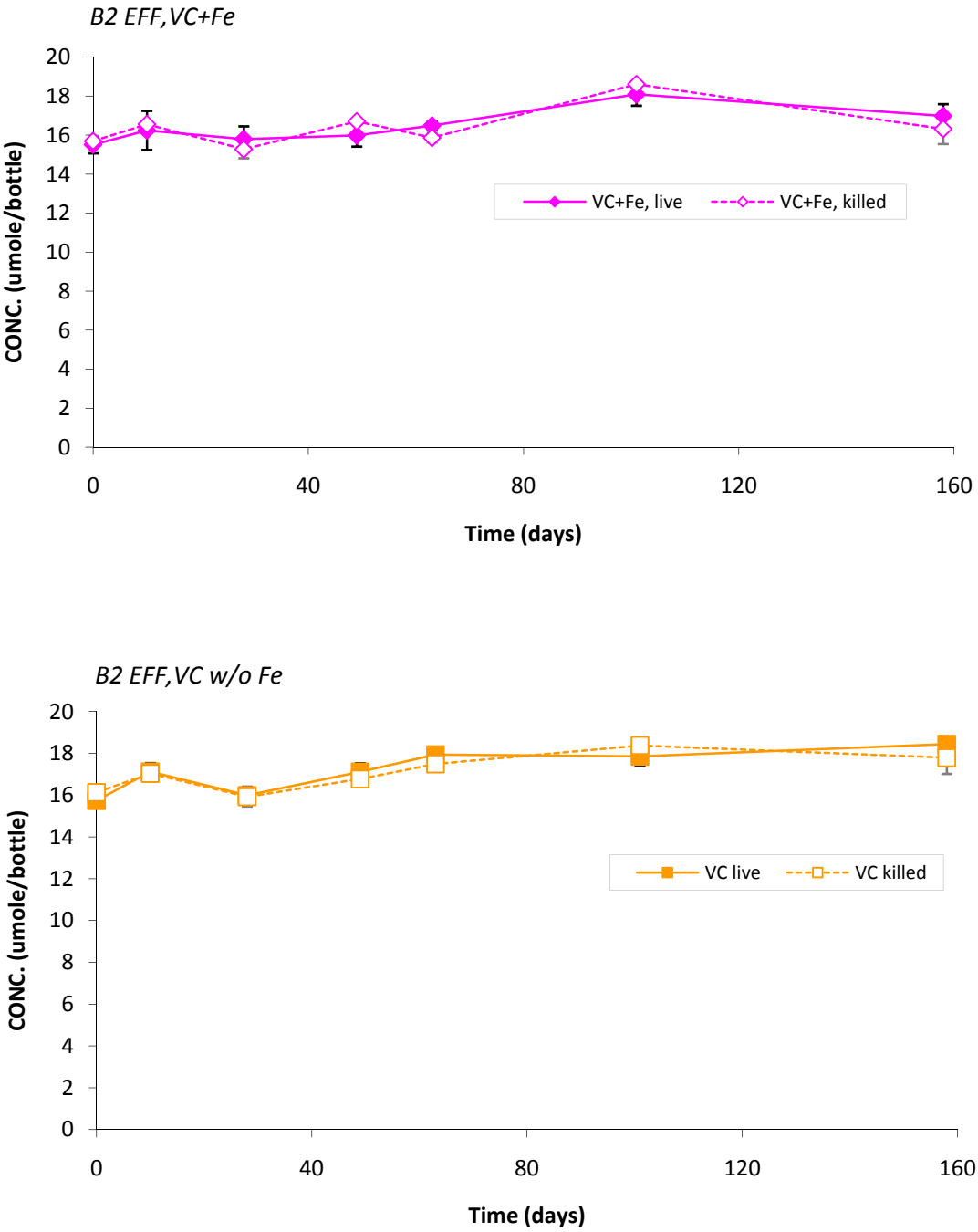


Figure 4.1.b Anaerobic microcosm results for Altus AFB Column B2: VC in VC-fed bottles.

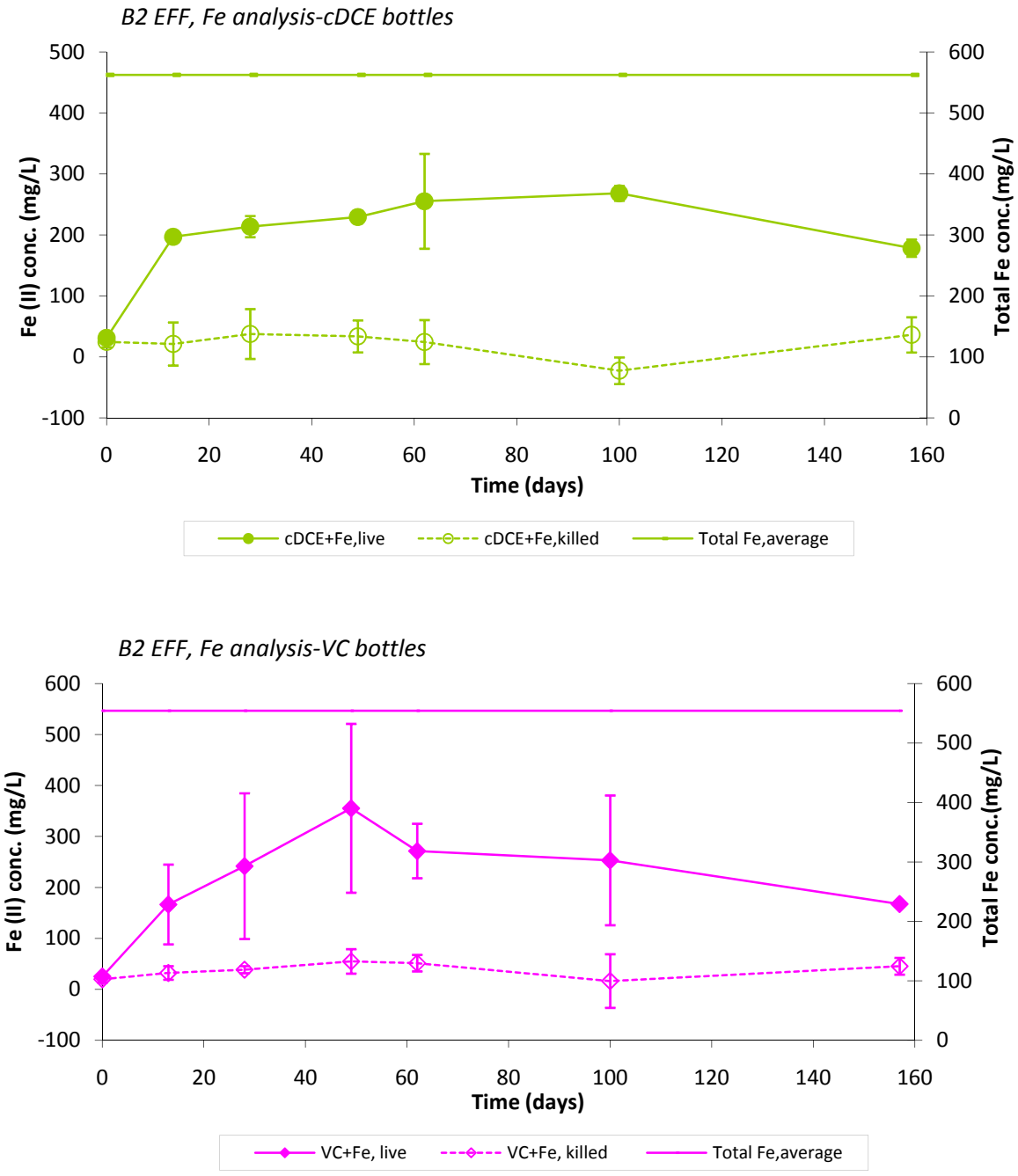


Figure 4.1.c Anaerobic microcosm results for Altus AFB Column B2: Fe Analysis.

4.1.1.2 Column B4

Following 152 days of incubation, no loss of cDCE or VC was observed in any microcosms constructed from column B4 material, except for one cDCE-fed bottle without amendment of Fe(III) , in which complete reductive dechlorination occurred (Figure 4.2 and Appendix III: 1-B).

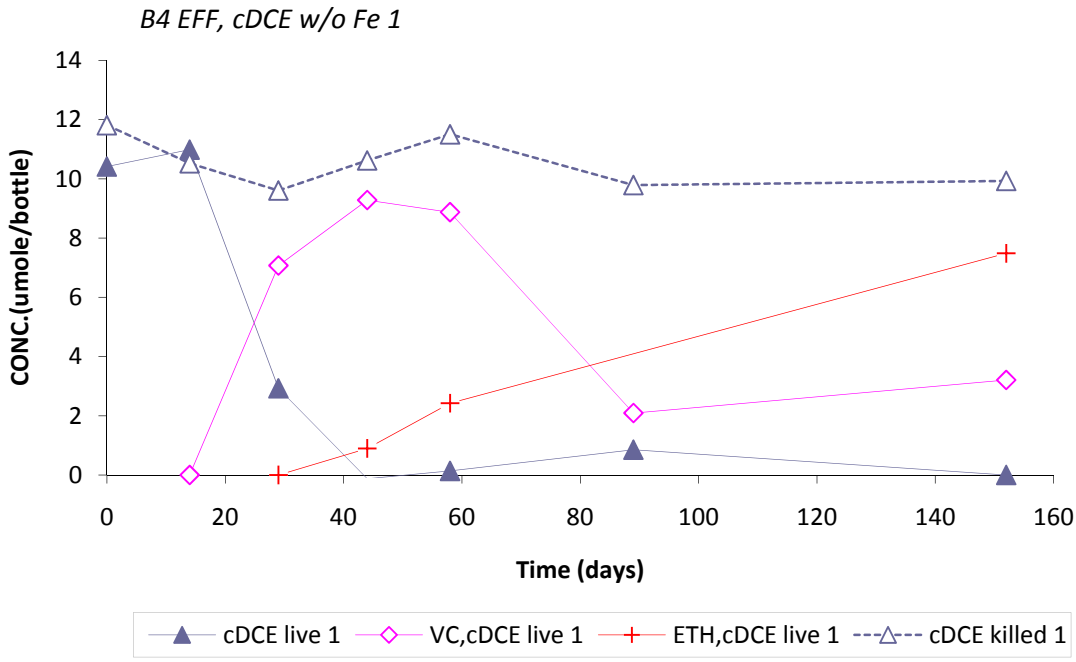


Figure 4.2 Anaerobic microcosm results for Altus AFB Column B4: Chloroethenes in cDCE-fed live 1 w/o Fe.

After a lag period of 14 days, VC started to increase with the corresponding decrease of cDCE. While 10.4 µmol cDCE was completely consumed by day 44, VC reached a highest level of 9 µmol and 0.9 µmol of ethene was formed. VC then declined between days 58 and 89, after which it remained at 2-3 µmol. The decrease of VC was concomitant with the continuous increase of ethene, which reached a final level of 7.5 µmol/bottle at day 152. (Note that ethene data was lost for day 89 so that it is not presented on the graph.) These results indicate a stoichiometric reductive

dechlorination of cDCE to VC and ethene in this microcosm. Transformation of cDCE can be ascribed to biological activity because no consumption of cDCE was observed in the autoclaved control. That reductive dechlorination of cDCE only happened in one of the two duplicate "cDCE live" bottles may be due to the fact that the materials in the microcosms were not homogeneous. The average total iron concentrations in cDCE and VC bottles amended with Fe(III) were 619 mg/L and 614 mg/L, respectively. Through 152 days of incubation period, Fe(II) concentrations in all Fe(III)-amended live bottles were between 100-200 mg/L, while about 100 mg/L Fe(II) was accumulated in the killed controls, suggesting that some of the Fe(II) production in these microcosms were abiotic. However, the reduction of Fe(III) did not stimulate the desired process of cDCE or VC oxidation.

4.1.2 Site/Source 2: Ithaca Area Wastewater Treatment Plant, Ithaca, New York

Results for 24 "sludge supernatant" microcosms are shown in Appendix III: 2-1A. Through 131 incubation days, reductive dechlorination occurred in one cDCE bottle with Fe(III) and one cDCE bottle without Fe(III). In the former case, trace amounts of VC (<0.25 μmol) started to appear in the cDCE-fed microcosm after a lag period of 71 days until the end of monitoring; in the latter case, 9 μmol cDCE was completely consumed and stoichiometrically transformed to VC via reductive dechlorination. The less quantities of dechlorination product (VC) observed in the Fe(III)-amended microcosm indicated that reductive dechlorination was effectively inhibited by the addition of Fe(III). No loss of cDCE or VC was observed in other microcosms, including those amended with Mn(IV). The average concentrations of total iron in the Fe-amended, cDCE-fed and VC-fed bottles were 531 mg/L and 555 mg/L, respectively. Fe(II) concentrations in these bottles first increased up to 170-260 mg/L by day 36, but dropped to 61-73 mg/L at day 131. Meanwhile, the original

orange color of the Fe(III)-amended microcosms disappeared, which was suggestive of complete conversion of Fe(III) to Fe(II). Accompanying this, however, was a decrease in measured total iron concentration to approximately 215 mg/L. The loss of measured Fe(II) and of total iron might be an artifact of sampling – i.e., due to precipitation or fixation on the walls of the bottles. Total Mn concentration in the Mn-amended, cDCE-/VC-fed bottles were 173 mg/L and 168 mg/L, respectively. However, the amount of Mn(II) formed did not increase above a background value, indicating that Mn(IV) reduction was not well established in these microcosms.

4.1.3 Site/Source 3: Plattsburgh AFB, New York

4.1.3.1 Influent to the Groundwater Treatment Plant

No degradation of cDCE, either by reductive dechlorination or anaerobic oxidation, was observed in any of the cDCE-fed bottles during 219 days of incubation. In VC-fed live bottles unamended with Fe(III), reductive dechlorination occurred (Figure 4.3). Ethene began to increase from day 41 and reached 9.9 μmol/bottle at day 219, while VC decreased from 25.3 to 18.6 μmol/bottle — a good mass balance of ethenes was achieved. No reductive dechlorination was observed in any VC-fed bottles amended with Fe(III), which might be because reductive dechlorination was suppressed by the addition of Fe(III). The average total iron concentrations in Fe-amended, cDCE- and VC-fed microcosms were 634 mg/L and 606 mg/L, respectively. Although Fe(II) was formed in both cDCE- and VC-fed bottles, it did not stimulate the biodegradation of cDCE or VC.

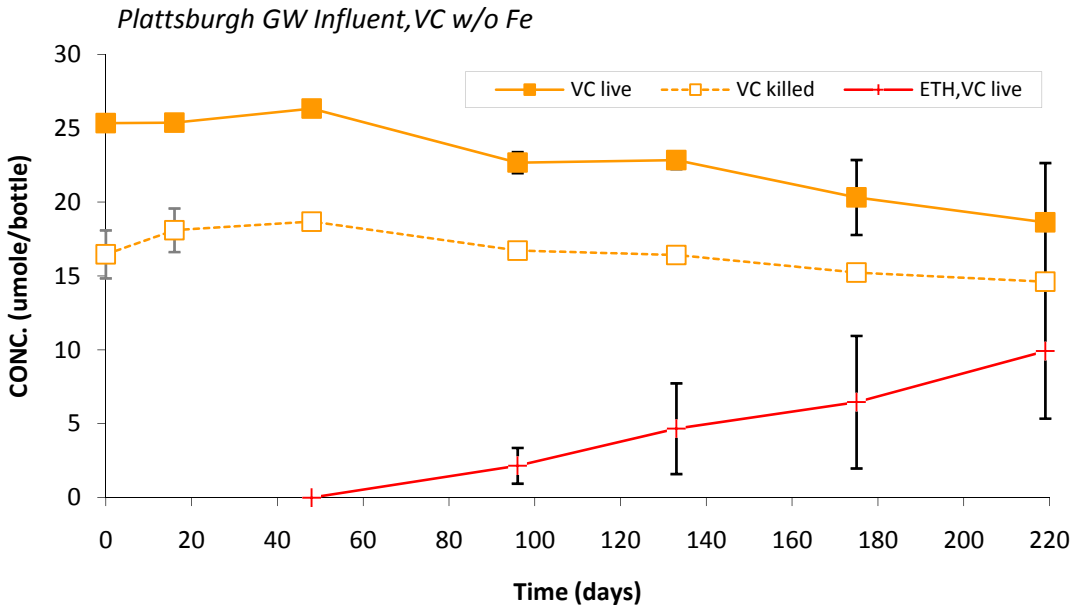


Figure 4.3 Anaerobic microcosm results for Plattsburgh AFB, Influent to the ground water treatment plant: Chloroethenes in VC live bottles w/o Fe.

4.1.3.2 Idaho Avenue Discharge Pipe

Microcosms prepared from material from the Idaho Ave location at Plattsburgh exhibited similar behavior to that discussed in the preceding section. In 16 microcosms of this series, no degradation of cDCE was detected, while VC consumption, via reductive dechlorination, occurred in both Fe(III)-unamended bottles. (Appendix III: 3-B). Following 173 days of incubation, approximately 4.7 µmol of ethene was accumulated, starting from day 41 with the simultaneous decrease of VC from 17.9 to 11.9 µmol. The decomposition of VC can be accredited to biological activity, since VC remained at the same level in the autoclaved controls. There appeared to be a 2-3 µmol loss of cDCE/VC in other microcosms (live and killed), which was most likely a consequence of leakage of chloroethenes through the bottles' septa after numerous punctures. Total iron concentrations in the designated cDCE and VC bottles were 558 mg/L and 620 mg/L, respectively. Despite that Fe(II) was accumulated in these bottles

(102 mg/L for cDCE-fed bottles; 78 mg/L for VC-fed bottles), it apparently did not result in the degradation of cDCE or VC.

4.1.3.3 East Flightline Discharge Pipe

Though 172 days of incubation, reductive dechlorination of cDCE to VC was observed in one cDCE-fed live bottle without Fe, in which VC started to form at day 20 and remained almost the same (6.7-7.3 μmol) beyond day 98. Minor reductive dechlorination of VC to ethene was detected in one VC-fed live bottle without Fe, where ethene began to accumulate following a lag period of 98 days and reached 1.2 μmol by day 172. No degradation of cDCE or VC occurred in other microcosms. However, the remaining amount of cDCE/VC in these microcosms (both live and killed) was more or less decreased, which was most probably a result of leakage losses. Initial concentrations of total iron in Fe-amended, cDCE- and VC-fed microcosms were 650 mg/L and 700 mg/L, and about 93 mg/L and 80 mg/L Fe(II) were formed at the last measurement point, respectively. The addition of Fe(III) did not stimulate the oxidation of cDCE or VC and probably suppressed the dechlorination process.

4.1.4 Site/Source 4: NAS Kings Bay, Georgia

4.1.4.1 Kings Bay KBA-11-13A

At the completion of 182-day monitoring, no loss of cDCE or VC was found in any of the 16 microcosms (Appendix III: 4-A). Neither was Fe(III) reduction well developed, as indicated by the low value of Fe(II) concentration measured (about 20 mg/L for both cDCE and VC bottles) at day 155.

4.1.4.2 Kings Bay Outcrop

At the completion of 172-day incubation, none of the 16 microcosms exhibited activity — either with respect to cDCE/VC consumption or Fe(II) accumulation (Appendix III: 4-B).

4.1.5 Site/Source 5: NAS Jacksonville, Florida

After 179 days of incubation period, all 16 microcosms showed no signs of cDCE or VC degradation (Appendix III: 5-A). Only a small amount of Fe(II) (43 mg/L and 80 mg/L in cDCE-fed and VC-fed bottles, respectively) accumulated in Fe(III)-amended microcosms, a large percentage of which was due to abiotic effects because minor accumulation of Fe(II) was also observed in the killed controls.

4.1.6 Site/Source 6: Undisclosed Superfund Sites

4.1.6.1 Series #1

During 186 days of incubation, no consumption of cDCE or VC was detected in any of the 16 microcosms (Appendix III: 6-A). The small decrease of chloroethenes measured at the last point was most likely due to leakage losses through the serum vials' septa. Total iron data were not shown on the "Fe analysis" graphs because total iron concentrations for these microcosms were above the upper limit of the FerroZine method we used (1.99-6.62 mg/L vs. 1.40 mg/L Fe). No attempt was made to requantify the amount of total iron since no significant amount of Fe(II) was formed and no degradation of cDCE or VC occurred in these microcosms. Although Fe(II) concentrations reached their highest level at the third measurement point, they did not reflect the real concentrations of Fe(II) in these bottles. As mentioned in "Materials and Methods", site materials from this Superfund site contained plenty of suspended particles, which resulted in variable absorbance readings highly affected by

the rate and time of centrifugation. This also explained why relatively high concentrations of Fe(II) were observed in the autoclaved controls. Filtration was employed at the last point of Fe(II) measurement, which suggested either that Fe(III) reduction was not well established in these microcosms, or that precipitation of Fe(II) species occurred.

4.1.6.2 Series #2

Of the 16 microcosms in this series, minor reductive dechlorination was observed in one cDCE-fed live bottle unamended with Fe(III) (Appendix III: 6-B). After a short lag period of 27 days, VC started to accumulate but remained at a low level that was less than 0.55 μmol/bottle at the completion of monitoring. cDCE level in the same microcosm, however, dropped from 10.7 to 7.9 μmol, which was most probably related to leakage losses. In one VC-fed live bottle without Fe(III), reductive dechlorination occurred. About 6 μmol of VC (from 20 to 14 μmol) was consumed, while ethene rose to 3 μmol. The difference between VC degradation and ethene production was most likely a result of leakage losses after numerous punctures. Additionally, VC level in the corresponding killed control, as well as other microcosms, more or less decreased probably due to the same reason. The average total iron concentrations in the Fe-amended, cDCE- and VC-fed bottles were 248 mg/L and 239 mg/L, respectively. Despite that approximately 96 mg/L and 117 mg/L Fe(II) were accumulated in the cDCE and VC microcosms correspondingly, Fe(III) reduction did not successfully induce anaerobic oxidation of cDCE or VC, when reductive dechlorination was completely inhibited.

4.1.6.3 Series #3-(a)

Following 185 days of incubation period, no consumption of cDCE/VC was detected in any of 16 microcosms (Appendix III: 6-C). However, the amount of chloroethenes decreased, to some extent, in all microcosms including the autoclaved

controls, which was most likely a consequence of leakage losses. Total iron concentrations in Fe-amended cDCE- and VC-fed microcosms were 695 mg/L and 650 mg/L, respectively. Fe(II) concentrations in these Fe(III)-amended bottles continuously increased up to 215-259 mg/L at day 147, where sufficient Fe(III) was still left as electron acceptor. Nevertheless, Fe(II) dropped to below 100 mg/L at the last measurement point on day 230, which might have resulted from the precipitation of Fe(II) species.

4.1.6.4 Series #3-(b)

Through 219 days of incubation, no degradation of cDCE or VC was observed in any of 16 microcosms (Appendix III: 3-D). The amount of chloroethenes was more or less lower than what was added initially, which was most probably due to leakage losses. The average total iron concentrations in Fe-amended, cDCE- and VC- fed microcosms were 576 mg/L and 606 mg/L, respectively. Only about 30 mg/L Fe(II) accumulated in the cDCE-fed microcosms, indicating that Fe(III) reduction was not well developed in these bottles. In the VC-fed microcosms, Fe(II) concentration first increased to 104 mg/L within 101 days, and then dropped to 29 mg/L on day 184, which might be a consequence of Fe(II) precipitation.

4.1.6.5 Series #3-(c)

At the completion of 197 days of incubation, all 16 microcosms showed no signs of cDCE or VC consumption (Appendix III: 6-E). Only 23 mg/L of Fe(II) accumulated in cDCE with Fe(III) live microcosms at the last measurement point (day 184), suggesting that Fe(III) reduction was not well established in these microcosms. Approximately 77 mg/L Fe(II) were accumulated in Fe-amended, VC-fed live bottles, but it did not stimulate the desired process of VC mineralization.

4.1.7 Site/Source 7: Cardinal Landfill, Farmington, New Hampshire

4.1.7.1 SO_4-1A+SO_4-2A

During 200 days of incubation, only one "cDCE-fed live without Fe(III)" microcosm showed minor reductive dechlorination (Appendix III: 7-A). Following a 134-day lag period, VC began to appear in the microcosm, but only 0.3 μmol of VC accumulated at the completion of monitoring. No loss of VC/cDCE was observed in other microcosms. The average total iron concentrations in Fe-amended, cDCE- and VC-fed microcosms were 614 mg/L and 626 mg/L, respectively. Less than 50 mg/L Fe(II) were accumulated in these microcosms by the last measurement on day 186.

4.1.7.2 SO_4-3A

After 199 days of incubation, trace amounts of VC were detected at the last measurement in cDCE-fed live bottles amended with Fe(III) (Appendix III: 7-B). cDCE concentration decreased from 8.5 to 6.9 μmol/bottle, with the production of about 0.3 μmol/bottle VC. The difference between cDCE consumption and VC formation was most probably due to the leakage losses from the microcosm septa after numerous punctures because that the corresponding killed controls also showed minor loss of cDCE (from 9.2 to 7.7 μmol). Similarly, trace amounts of VC were also observed in cDCE-fed live bottles without Fe(III), following a lag period of 71 days. However, the concentration of VC remained below 0.2 μmol/bottle to the end of this microcosm study of the Cardinal site. No VC degradation occurred in VC-fed microcosms, either amended or unamended with Fe(III). The average total iron concentrations in cDCE and VC microcosms were 554 mg/L and 534 mg/L, respectively. After 185 days, less than 17.4 mg/L (38.0 minus 20.6 mg/L) Fe(II) was produced in Fe-amended cDCE-fed microcosms, while Fe(II) level did not increase in the VC-fed bottles.

4.1.8 Site/Source 8: Aberdeen Proving Ground, Maryland

4.1.8.1 WB 23

Vigorous reductive dechlorination occurred in all live microcosms of WB23, with or without Fe(III) (Figure 4.4). In Fe-amended, cDCE-fed live bottles, cDCE (7.7 μmol) was degraded entirely by day 33, with ethene (7.3 μmol) as the only detectable product. 150 μL of cDCE saturated solution was then respiked to the microcosms on day 34. Two μmol of VC had accumulated when headspace analysis was performed after the microcosms were agitated on an orbital shaker for an hour. cDCE, as well as its dechlorination product, VC, was rapidly depleted again within seven days, while ethene crested to approximately 14 μmol/bottle. These results suggested that the chloroethene precursors ((7.7 + 3.8) μmol cDCE + 2 μmol VC=13.5 μmol) were stoichiometrically converted to ethene via complete reductive dechlorination. cDCE-fed live bottles exhibited similar behavior as that in Fe(III)-amended microcosms. After the first addition of 7.3 μmol cDCE was fully degraded to 7.3 μmol of ethene, another 150 μL cDCE saturated solution was respiked to the microcosms. Within seven days, chloroethene precursors (9.5 μmol cDCE and 2 μmol VC) were depleted and converted to 7.5 μmol ethene. The difference between cDCE/VC consumption and ethene production might result from the leakage losses from the microcosm septa, which could also explain the phenomenon of cDCE decrease in the corresponding killed controls. In VC-fed live microcosms, reductive dechlorination exhibited similar rate and extent in the Fe(III)-amended and unamended bottles. During the first 33 days, about 20 μmol of VC was biotransformed to approximately 16 μmol of ethene. The second addition of about 17 μmol VC was depleted rapidly in 7 days, with accumulation of about 12 μmol ethene. Total iron concentration in cDCE-fed, Fe-amended microcosms were 475.4 mg/L, and about 251.8 mg/L of Fe(II) was produced by day 40; total iron in VC-fed bottles was 583.4 mg/L, and about 222.9 mg/L Fe(II)

was accumulated by day 47. The iron analysis results showed that Fe(III) reduction was well developed in these microcosms. However, the addition of Fe(III) did not inhibit the occurrence of reductive dechlorination, either in terms of degradation rate or extent. Furthermore, a large methane output was detected in all the cDCE-and VC-fed microcosms (data not shown), suggesting that methanogenesis was not completely suppressed by the Fe(III). Since the wetland sediment obtained from Aberdeen site was rich in organic matter, which could provide abundant sources of electron donors, competition for reducing equivalents between reductive dechlorinators and other metabolic groups of indigenous microorganisms did not become a limiting factor for the occurrence of reductive dechlorination. Because the organic-rich, highly reducing environment would not support anaerobic oxidation of cDCE and VC, monitoring was ceased after 47 days.

4.1.8.2 WB 30

Microcosms for WB 30 exhibited similar activity as that in WB 23 microcosms — extensive reductive dechlorination was detected in all live bottles amended and unamended with Fe(III) (Appendix III: 8-B). In cDCE-fed live bottles (with and without Fe), about 5 μmol added cDCE was consumed, with about 7.5 μmol ethene as the only detectable product after 32 days. Likewise, in VC-fed live microcosms, an average of approximately 80% of the VC consumption could be attributable to ethene production by day 32 (18.8-19.2 μmol VC vs. 15 μmol ethene). The dechlorination of cDCE/VC to ethene was accompanied by a large output of methane observed in all the live microcosms (data not shown), suggesting that methanogenesis was not inhibited by the addition of Fe(III). Total iron and Fe(II) were only measured at the beginning when microcosms were constructed. No attempt was made to measure the subsequent change of Fe(II) in these microcosms because anaerobic oxidation would not be

favorable under highly reducing methanogenic conditions. Monitoring was then terminated after day 32.

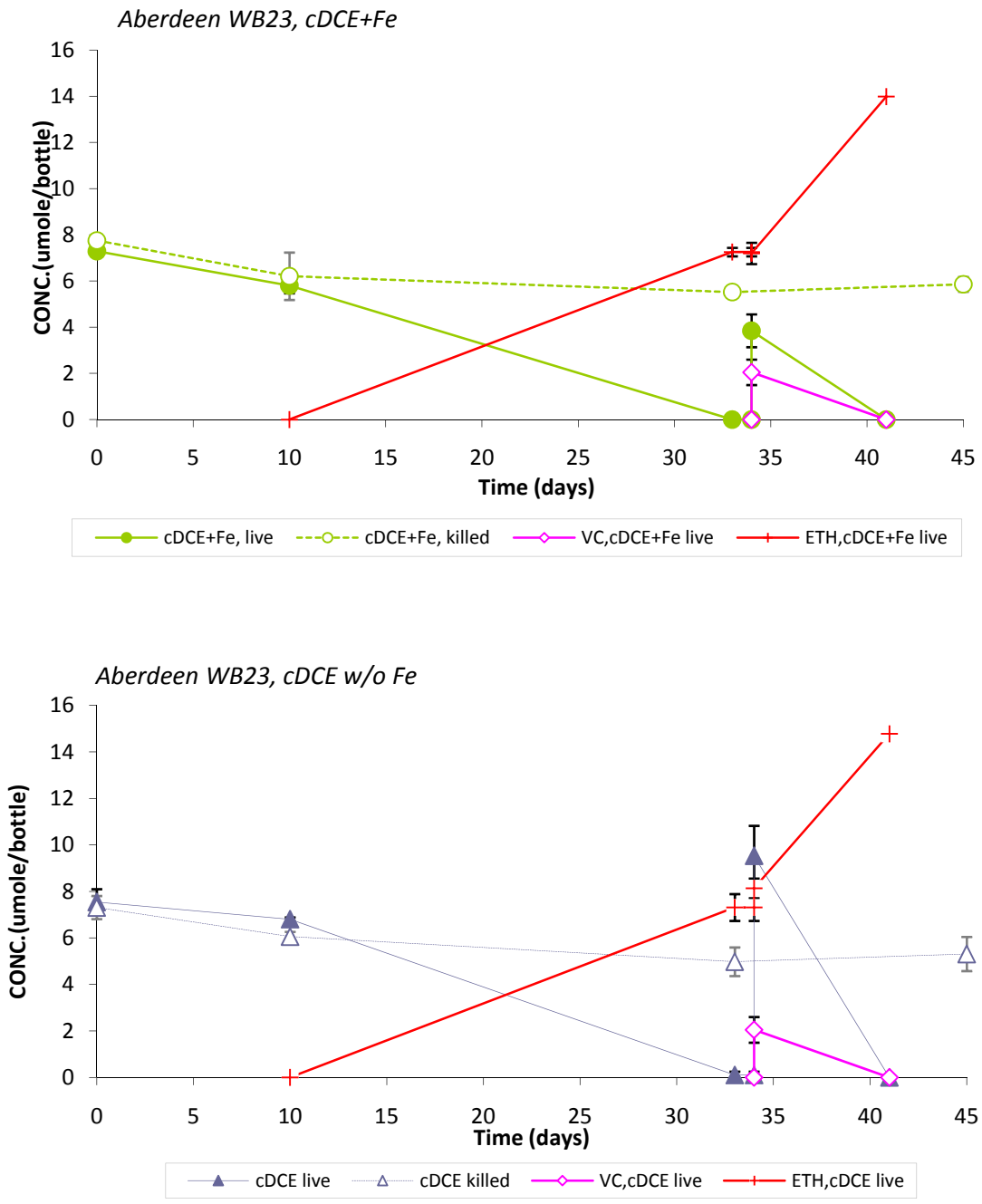


Figure 4.4.a Anaerobic microcosm results for Aberdeen WB23: cDCE in cDCE-fed bottles.

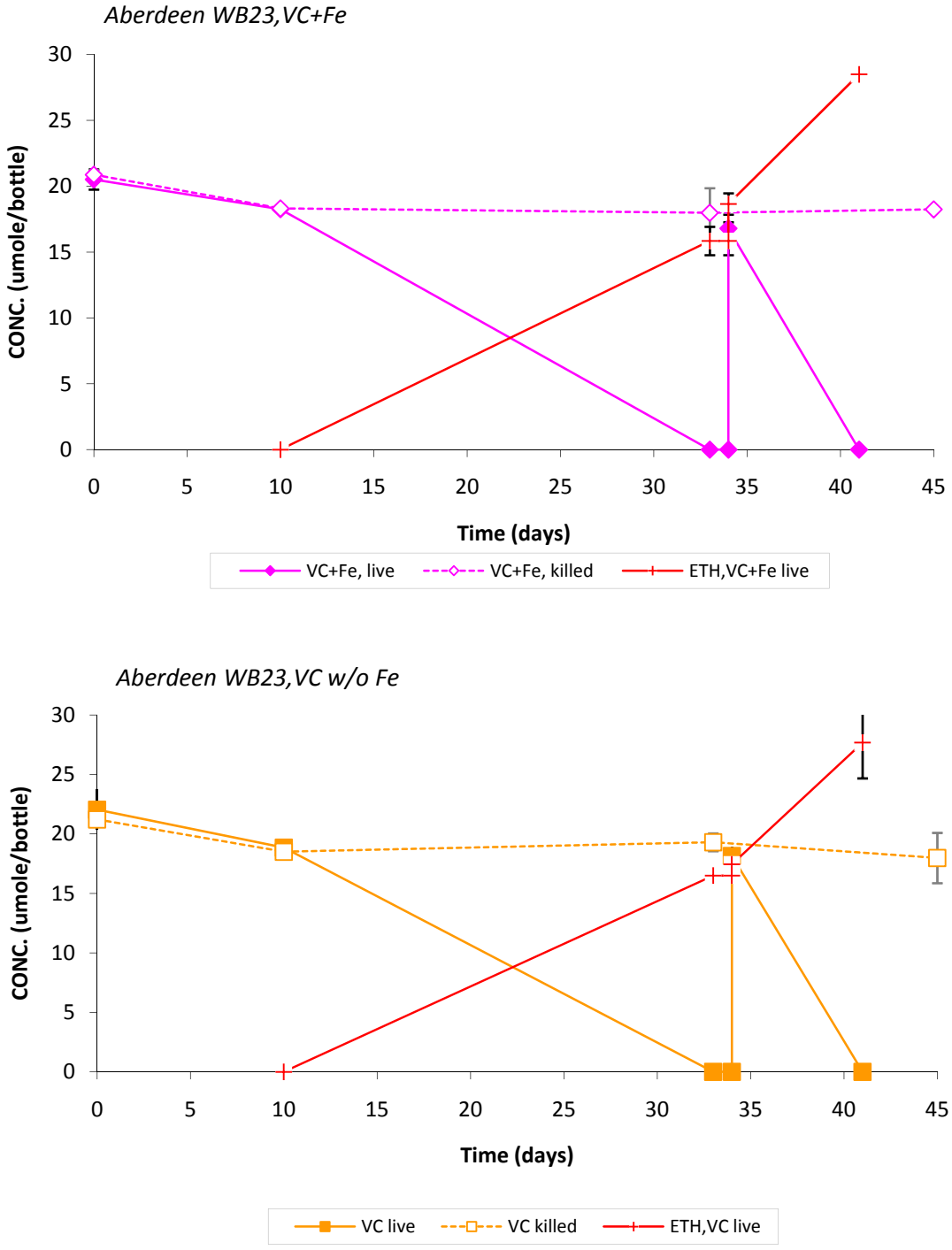


Figure 4.4.b Anaerobic microcosm results for Aberdeen WB23: VC in VC-fed bottles.

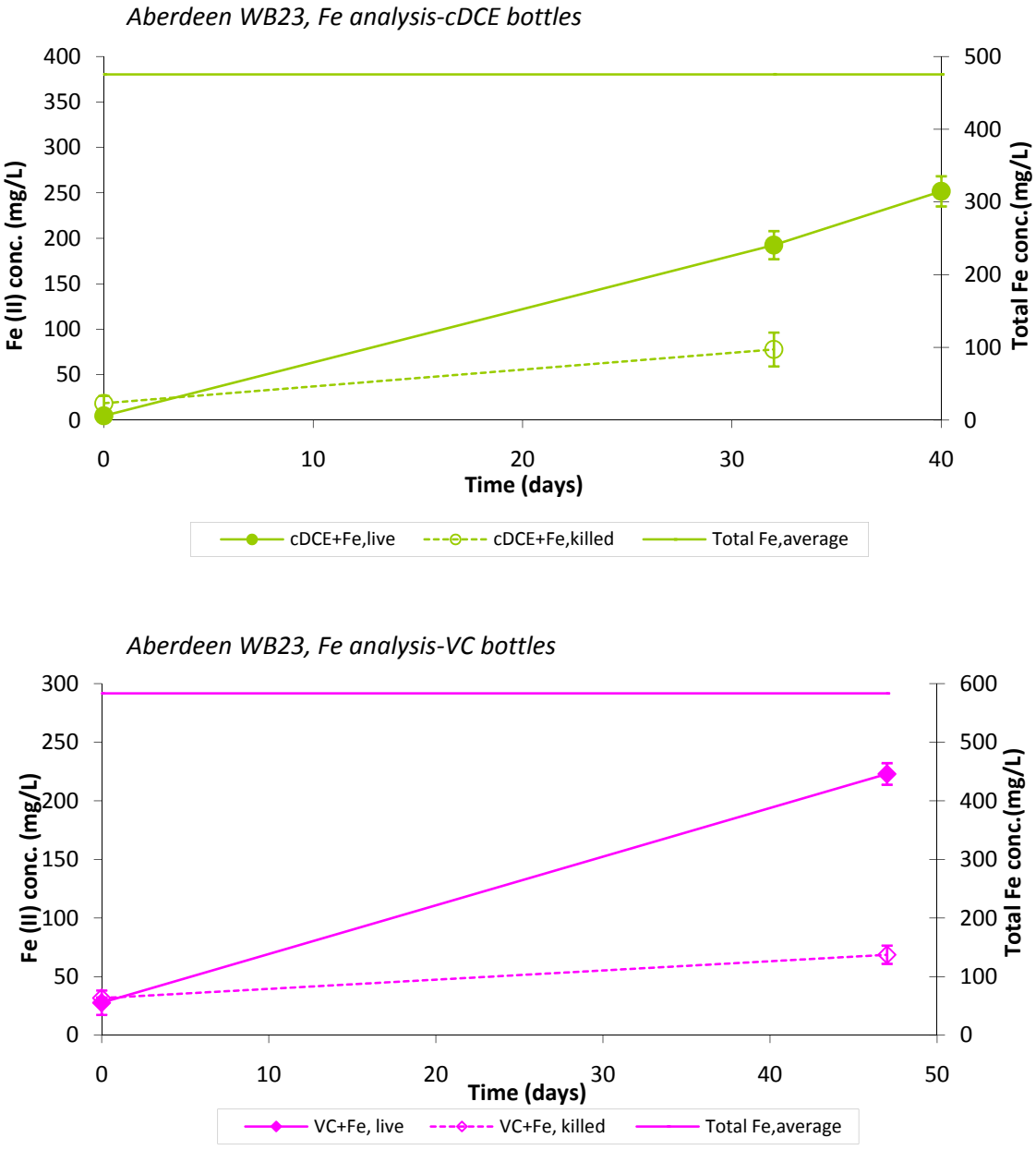


Figure 4.4.c Anaerobic microcosm results for Aberdeen WB23: Fe analysis.

4.1.9 Site/Source 9: NAS Cecil Field, Florida

4.1.9.1 Sediment+Groundwater

Following 135-148 days of incubation, significant cDCE and VC consumption, via anaerobic reductive dechlorination was observed in all 8 live microcosms, but the rate and extent of dechlorination differed between duplicate microcosms, as well as between bottles with and without Fe(III) (Figure 4.5). In cDCE-fed live microcosms amended with Fe(III), one exhibited minor reductive dechlorination — 0.9 μmol of VC was accumulated during the 135-day incubation, while 3.6 μmol of cDCE was degraded (from 7.5 to 3.9 μmol). The lack of mass balance of chloroethenes was probably due to leakage, since in its duplicate microcosm, the concentration of cDCE declined with the coincident accumulation of VC and ethene after day 9. VC, which had increased to 5.3 μmol, started to decrease after day 100, while ethene kept rising till reached 8.7 μmol. On day 135, approximately 82.1% of cDCE was converted to ethene. The loss was most likely a consequence of leakage from the microcosm septa after numerous punctures, as also indicated by the corresponding autoclaved control. In comparison with microcosms amended with Fe(III), one cDCE-fed live bottle without Fe(III) showed complete reductive dechlorination from cDCE to VC to ethene, followed by further reduction of ethene to ethane. VC started to accumulate in the microcosm without a lag, accompanied by the simultaneous consumption of cDCE. Ethene appeared after day 9 and kept rising till reached the highest level of 11 μmol/bottle on day 69, when cDCE and VC were almost depleted while ethene began to convert to ethane. At the last measurement point, day 148, all the 11 μmol of formerly accumulated ethene was completely reduced to 12.5 μmol of ethane — a fairly good mass balance of ethenes was achieved. The duplicate cDCE-fed bottle was

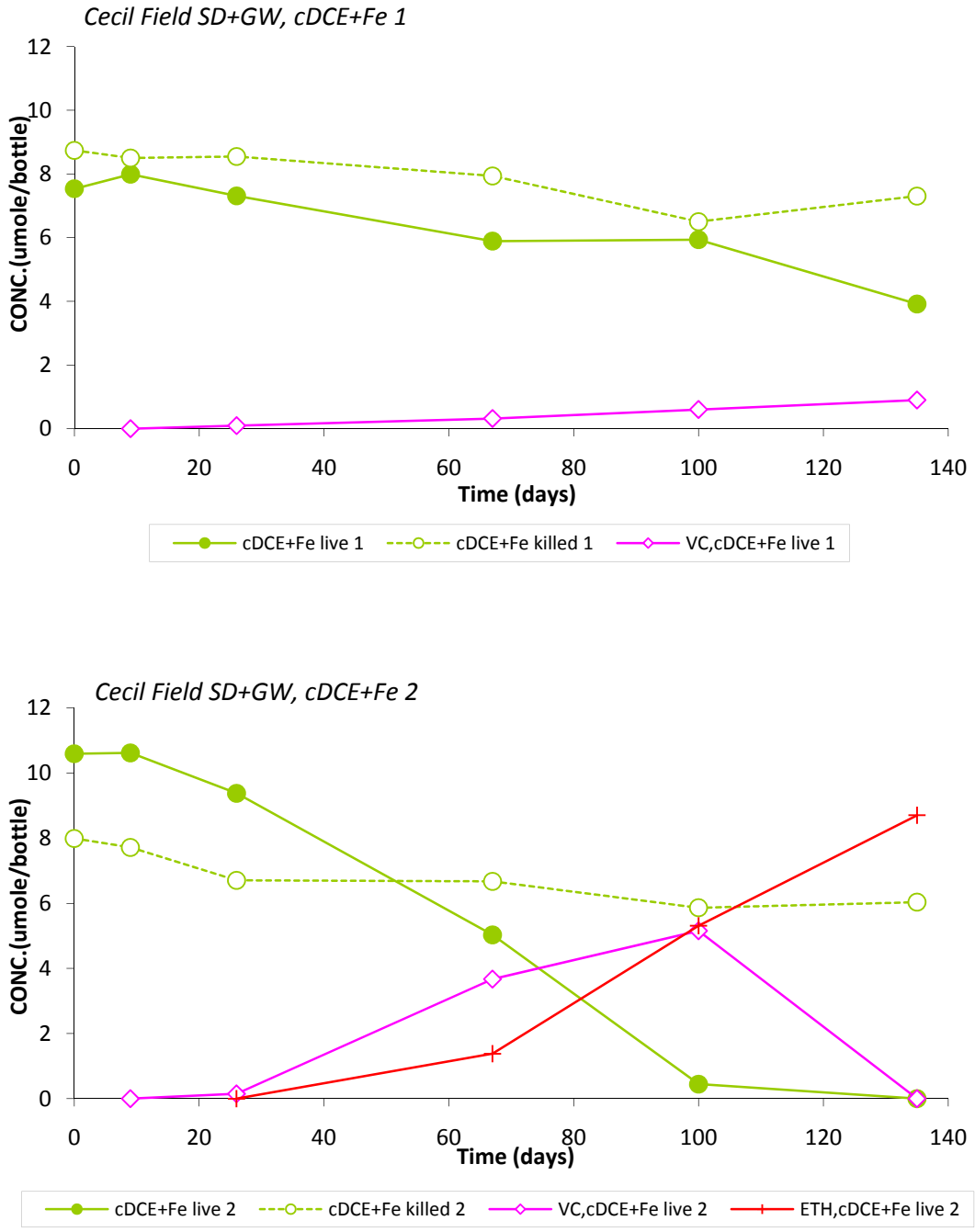


Figure 4.5.a Anaerobic microcosm results for Cecil Field sediment+groundwater: Chloroethenes in cDCE+Fe microcosms.

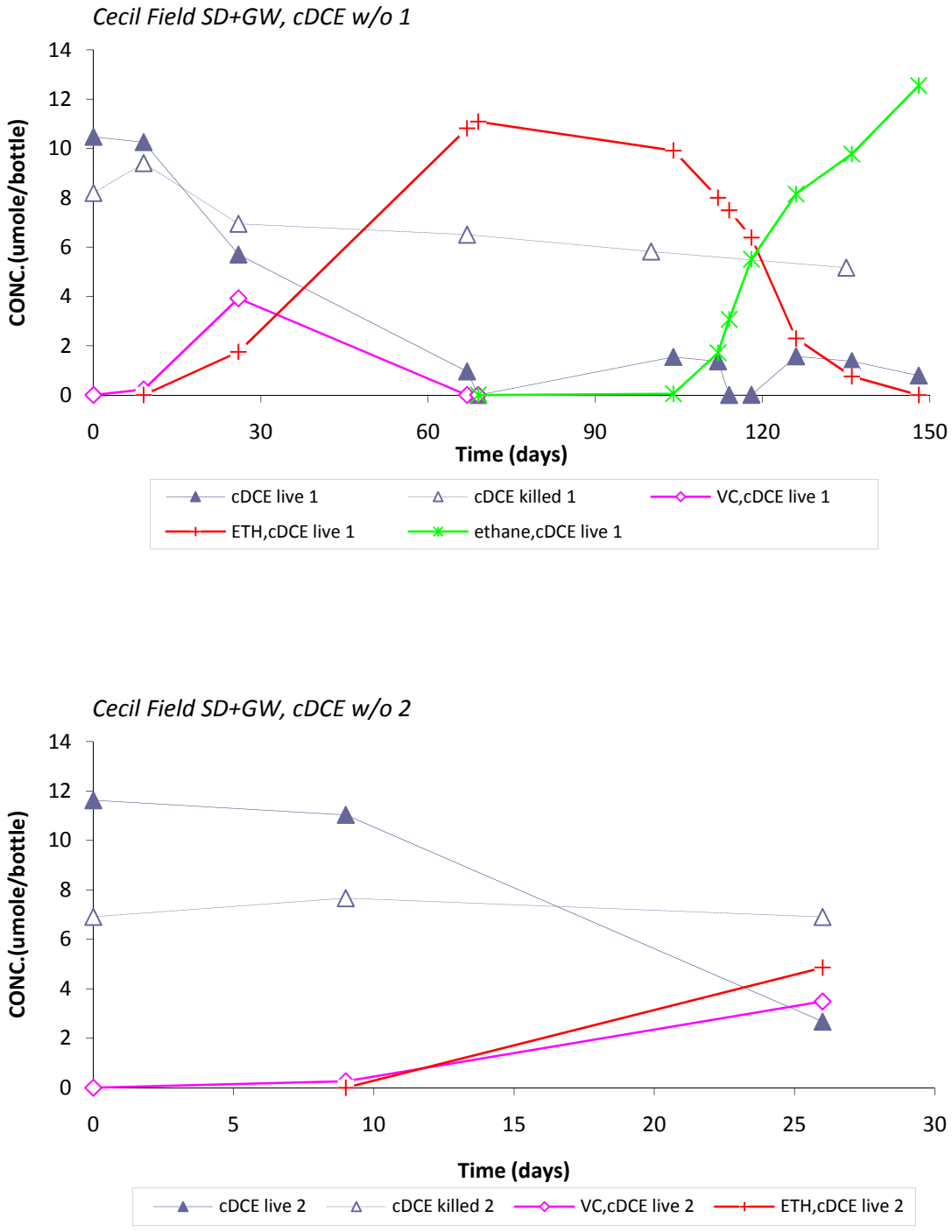


Figure 4.5.b Anaerobic microcosm results for Cecil Field sediment+groundwater: Chloroethenes in cDCE w/o Fe microcosms.

removed for subsequent aerobic studies (described previously in "Materials and Methods") on day 26, when reductive dechlorination products – VC and ethene – had accumulated. Ethane was also found as the end product of reductive dechlorination in two VC-fed live microcosms. In one of the VC-fed live bottles amended with Fe(III), VC was rapidly transformed to ethene by day 118, after which ethene started to decline while ethane rose. By day 135, all 18.5 μmol of VC was depleted, with accumulation of 9.4 μmol of ethene and 7.2 μmol of ethane, respectively. In contrast, minor reductive dechlorination occurred in the replicated VC with Fe(III) microcosm. Following a lag period of 100 days, 1.6 μmol of ethene was produced on day 135. In one of the VC-without-Fe(III) live microcosms, ethene started to accumulate immediately without a lag and kept increasing with the concomitant decrease of VC. When VC was entirely consumed on day 118, ethene began to decline while ethane started to appear in the microcosms. At the completion of monitoring (day 148), 18.6 μmol of ethane was the only detectable end product of reductive dechlorination. 18.6 μmol of added VC was stoichiometrically converted to ethane. The duplicate VC w/o Fe(III) bottle was taken out for the aerobic studies on day 26 when ethene had accumulated. The average total iron concentrations in Fe-amended, cDCE- and VC-fed microcosms were 552 mg/L and 573.7 mg/L. At last measurement on day 127, 101.9 mg/L of Fe(II) had accumulated in the cDCE-fed bottles; while 94.5 mg/L Fe(II) was formed in the VC-fed bottles. It is noteworthy that the complete reduction from ethene to ethane observed in the three microcosms was accompanied by a large production of methane, which gave evidence that the nutrient-rich materials from Cecil Field provided sufficient electron donors for the growth of both methanogens and Fe(III)-reducers.

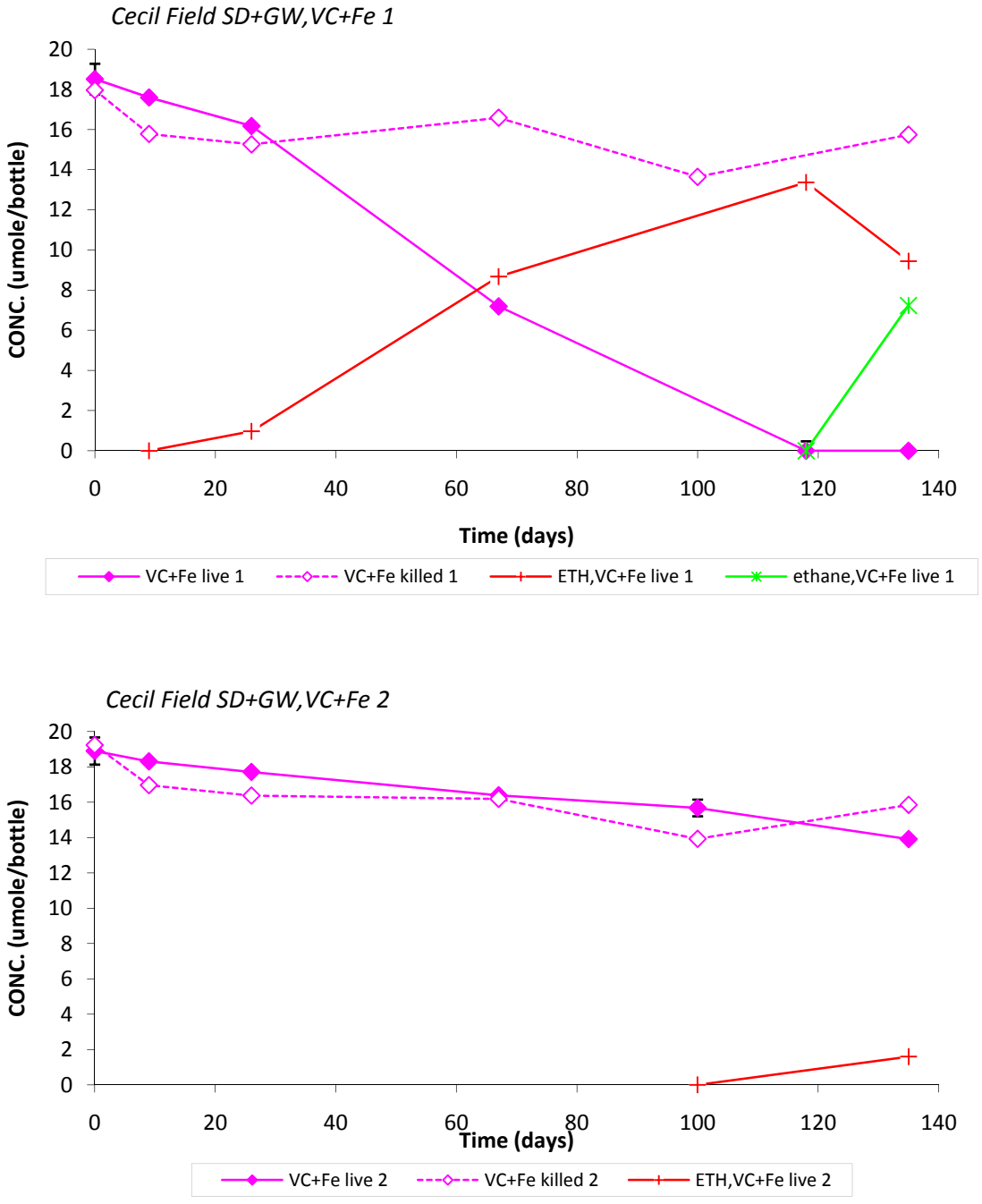


Figure 4.5.c Anaerobic microcosm results for Cecil Field sediment+groundwater: Chloroethenes in VC+ Fe microcosms.

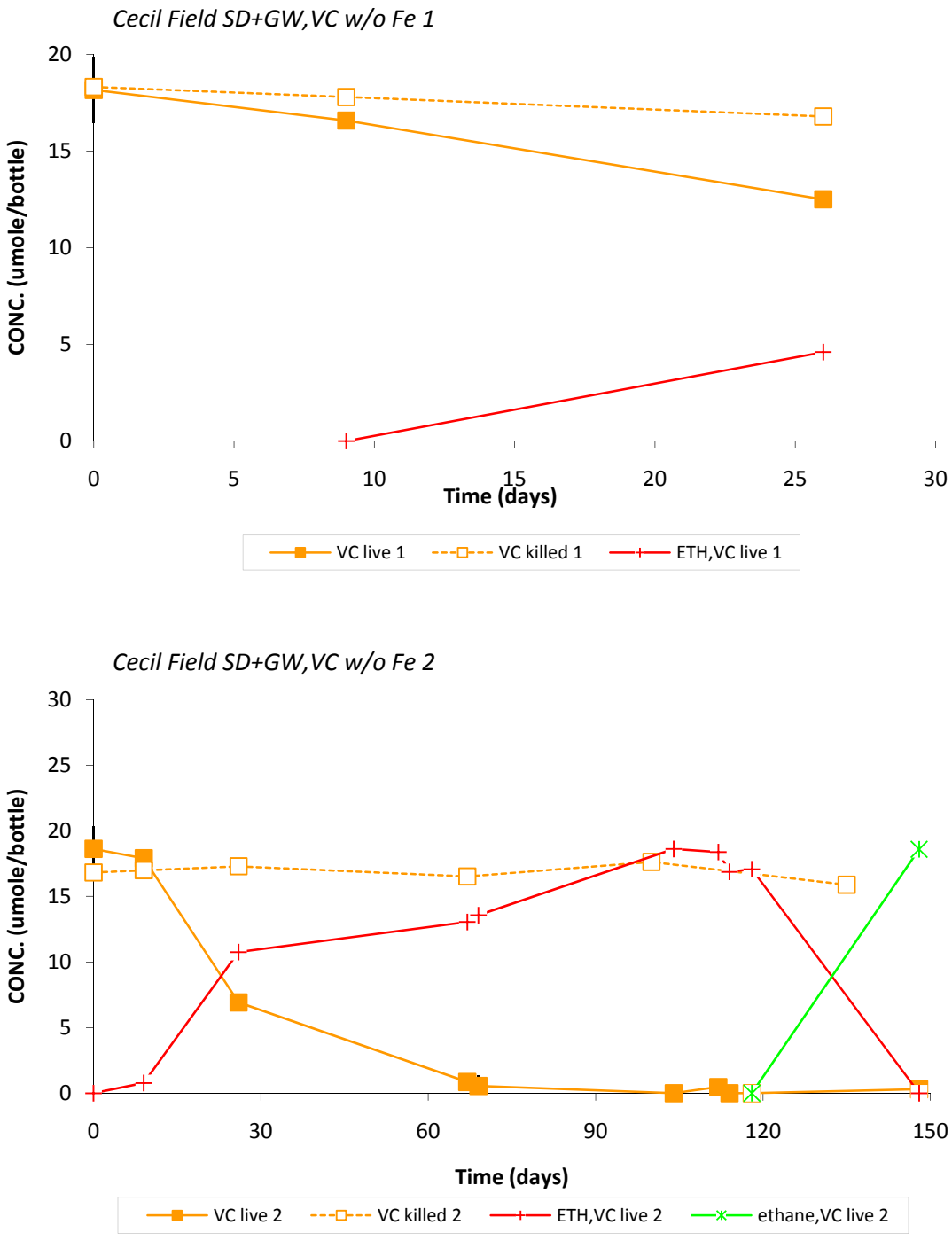


Figure 4.5.d Anaerobic microcosm results for Cecil Field sediment+groundwater: Chloroethenes in VC w/o Fe microcosms.

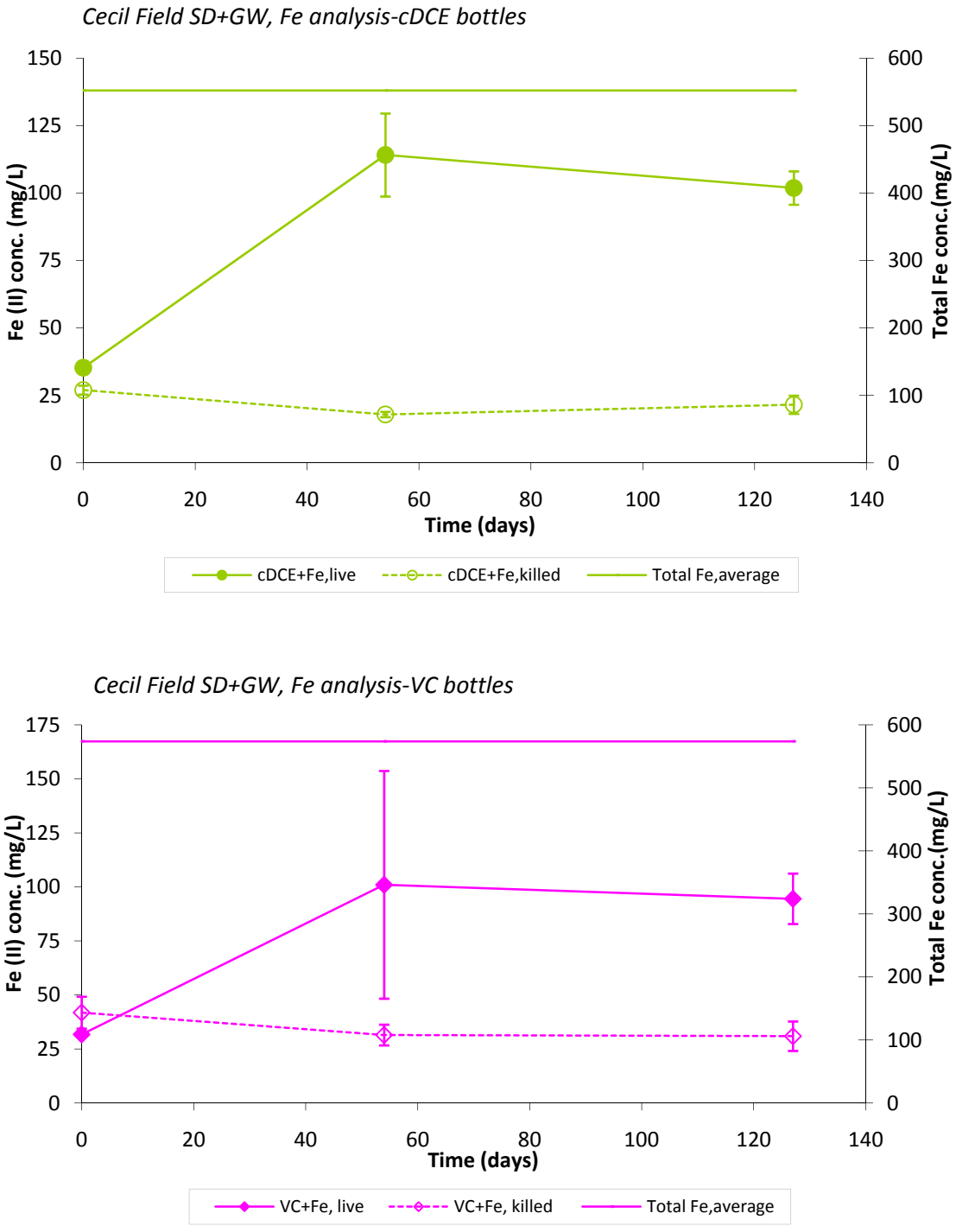


Figure 4.5.d Anaerobic microcosm results for Cecil Field sediment+groundwater: Fe Analysis.

4.1.9.2 Sediment+Groundwater+Medium

In all eight live microcosms, reductive dechlorination occurred, with VC, ethene or ethane as the final product (Appendix III: 9-B). The duplicate microcosms showed a degradation activity similar to each other in this series of Cecil Field microcosms. In Fe-amended, cDCE-fed live bottles, one showed 2.9-μmol degradation of cDCE with production of 3.5 μmol VC and 0.6 μmol ethene on day 119; the other one had 0.7 μmol of cDCE converted to 0.6 μmol of VC, which was the only detectable dechlorination product by day 119. The extent of reductive dechlorination was much more extensive in cDCE microcosms unamended with Fe(III), in which reduction from ethene to ethane was observed. In the first cDCE-w/o-Fe(III) live bottle, cDCE kept declining till the depletion on day 105, while ethene and VC were accumulated without a lag. On day 36, VC was almost entirely consumed, while ethene started to decrease with the concurrent increase of ethane production. For the last measurement, the addition of 5.1 μmol of cDCE was converted to 6.8 μmol of ethane. Ethane was also the end product of the replicate cDCE-w/o-Fe(III) live bottle, but appeared later after 90 days. At the last measurement point, about 6.0 μmol of cDCE was depleted with 7.4 μmol of ethane produced. In Fe-amended, VC-fed live microcosms, minor reductive dechlorination was observed. Following a lag period of 36 days, 0.9 μmol of ethene was accumulated in the first bottle on day 120; ethene started to appear after a 90-day lag period in the second bottle and increased to 1.2 μmol on day 120. In the first VC-w/o- Fe(III) live microcosm, ethene was the principal product — 23.7 μmol of VC degradation was completely accounted for the production of 26.6 μmol of ethene and 0.9 μmol of ethane. On the other hand, ethane was the predominant product in its replicated microcosm — 16.7 μmol of VC was completely converted to 18.2 μmol of ethane and 3.9 μmol of ethene during 120 days. The average total iron concentrations in Fe-amended, cDCE- and VC-fed microcosms

were 435.5 mg/L and 466.3 mg/L, respectively. Little accumulation of Fe(II) was detectable in the Fe(III)-amended microcosms after 95 days. However, the addition of Fe(III) did inhibit, to some extent, the occurrence of reductive dechlorination (more extensive reductive dechlorination happened in bottles without iron). Additionally, compared to the large output of methane in the live microcosms without Fe(III), only a small amount of methane was detected in the live microcosms amended with Fe(III) (data not shown).

4.1.9.3 Sediment+Medium

Given that robust reductive dechlorination occurred in the previously prepared Cecil Field microcosms due to the nutrient-rich nature of the site materials, no yeast extract was added to the basal medium when constructing the third series of microcosms. Following 84 days of incubation period, minor reductive dechlorination was observed in four cDCE-fed live bottles amended with Fe(III) or Mn(IV) — the production of VC or VC + ethene was below 1 μmol (Appendix III: 9-C). In contrast, complete reductive dechlorination occurred in live bottles without Fe(III) or Mn(IV), ethane accumulated in one of the duplicate microcosms. VC started to accumulate in these microcosms without a lag, and then decreased with the continuous production of ethene. By day 84, 94.3% of cDCE degradation could be attributable to the accumulation of ethene in the first microcosm (10 μmol ethene production vs. 10.6 μmol cDCE consumption). In its duplicate microcosm, cDCE and VC entirely disappeared with 8.6 μmol of ethene as the final product (7.4 μmol cDCE was added). Trace amount of ethane appeared on day 84. No degradation of VC was observed in VC-fed microcosms with Fe(III) or Mn(IV), while complete reductive dechlorination occurred in VC live bottles without Fe(III) and Mn(IV). At the completion of monitoring on day 84, an average of 89.9% of VC was converted to ethene (14.3 μmol ethene formed vs. 15.9 μmol VC added). Total iron data were not shown on “Fe

analysis" graphs because that total iron concentrations for these microcosms were above the upper limit of the FerroZine method we used (1.17-3.27 mg/L vs. 1.40 mg/L Fe). No attempt was made to requantify the amount of total iron since the only observed metabolic process in these microcosms was reductive dechlorination. During 61 days, approximately 40 mg/L of Fe(II) accumulated in the Fe(III)-amended microcosms (from 54.3 to 93.3 mg/L in cDCE-fed bottles; from 52.5 to 93.8 mg/L in VC-fed bottles); On day 73, the measured Mn(II) concentration was below 30 mg/L (similar values were measured in the killed controls), which suggested that Mn(IV) reduction was not well developed in these microcosms. Compared with the chloroethene results of Fe(III)- and Mn(IV)-unamended live microcosms, the addition of Fe(III) or Mn(IV) could contribute to the inhibition of reductive dechlorination, as well as the production of methane .

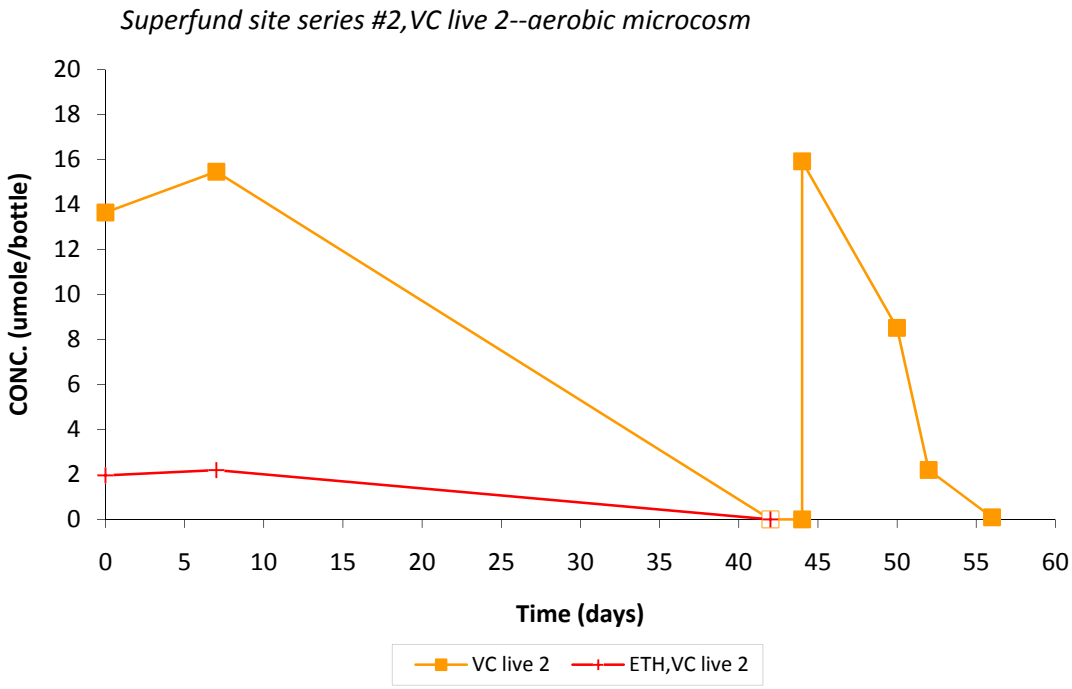
4.2 Results for Aerobic Microcosms

As outlined earlier, we hypothesized that what some have reported to be anaerobic oxidation might actually be aerobic oxidation occurring under conditions of low oxygen-flux at extremely low levels of dissolved oxygen. We thus investigated the potential for aerobic oxidizers to survive after prolonged incubation in our ostensibly anaerobic microcosms. Therefore, 10 mL oxygen was added to 16 of the formerly anaerobic microcosms from the following sites: Plattsburgh AFB, Influent to the groundwater treatment plant and Idaho Avenue discharge pipe; NSB Kings Bay, KBA-11-13A and Outcrop; Unidentified Superfund Site, Subset #1, Subset #2, and Subset #3-(a); NAS Cecil Field, sediment+groundwater. Microcosms for the aerobic experiment are shown in Appendix IV: Table IV. Most of these microcosms were unamended with Fe(III) and had shown no degradation of cDCE or VC during anaerobic incubation. The corresponding duplicate anaerobic microcosms continued to

serve as the anoxic controls for the aerobic experiment. The remaining amounts of chloroethenes and oxygen were measured periodically with GC/FID and GC/TCD, respectively.

After 64 days of aerobic incubation, no oxidation of cDCE was observed in any of the cDCE-fed microcosms. One cDCE-fed microcosm, constructed with sediment and groundwater from Cecil Field, showed extensive reductive dechlorination even after the addition of oxygen (Appendix IV: 1-A). Repeated additions of cDCE could be degraded without a lag. Although oxygen remaining in the aerobic microcosms was monitored periodically, there were two peaks that co-eluted with each other on the GC/TCD chromatogram for Cecil Field microcosm. These two peaks were first thought to be O_2 and CO_2, but turned out to be CO_2 and ethene after identification. Consequently, the occurrence of reductive dechlorination in the originally aerobic microcosm probably resulted from the depletion of oxygen by a range of degradation processes in the organic-rich materials from Cecil Field and the dominance of reductive dechlorinators thereafter.

Among eight of the VC-fed aerobic microcosms, three microcosms – from Undisclosed Superfund Sites Series #2 and #3(a) and Cecil Field – showed VC-oxidizing activity (Figure 4.6). VC, along with previously accumulated ethene, was completely degraded within 44 days. A subsequent addition of VC was entirely consumed in 12 days without accumulation of any reductive dechlorination products. In their anoxic controls, VC either remained more or less the same level or degraded via reductive dechlorination. No oxidation of VC was observed in other VC-fed aerobic microcosms (Appendix IV: 1-B).



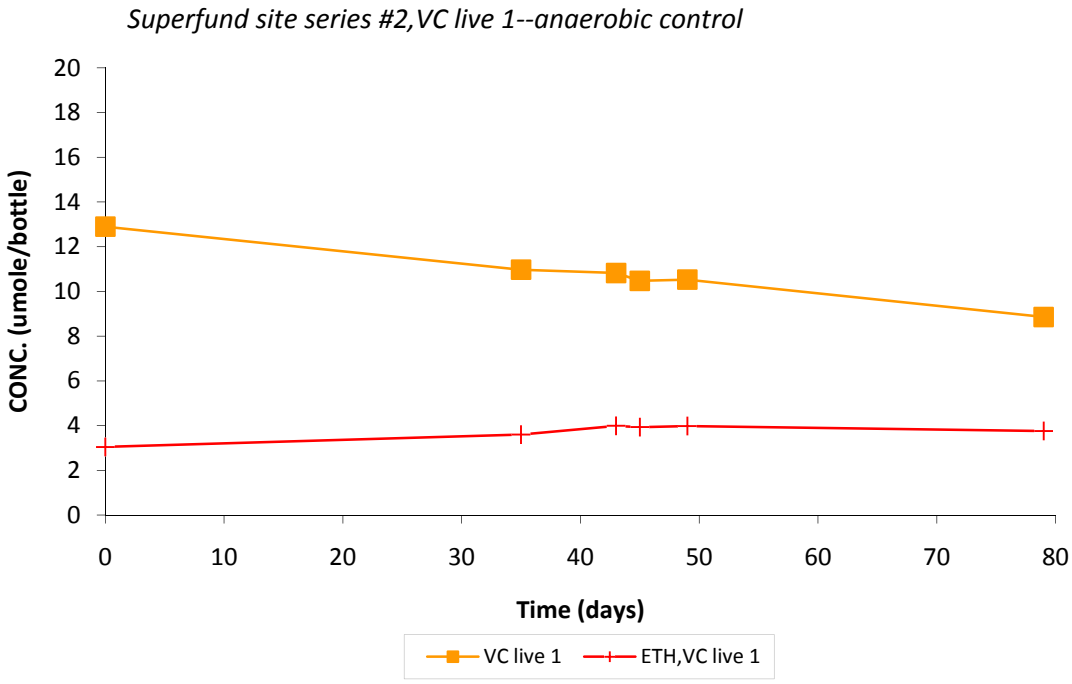


Figure 4.6.a Aerobic microcosm results for Superfund site series #2.

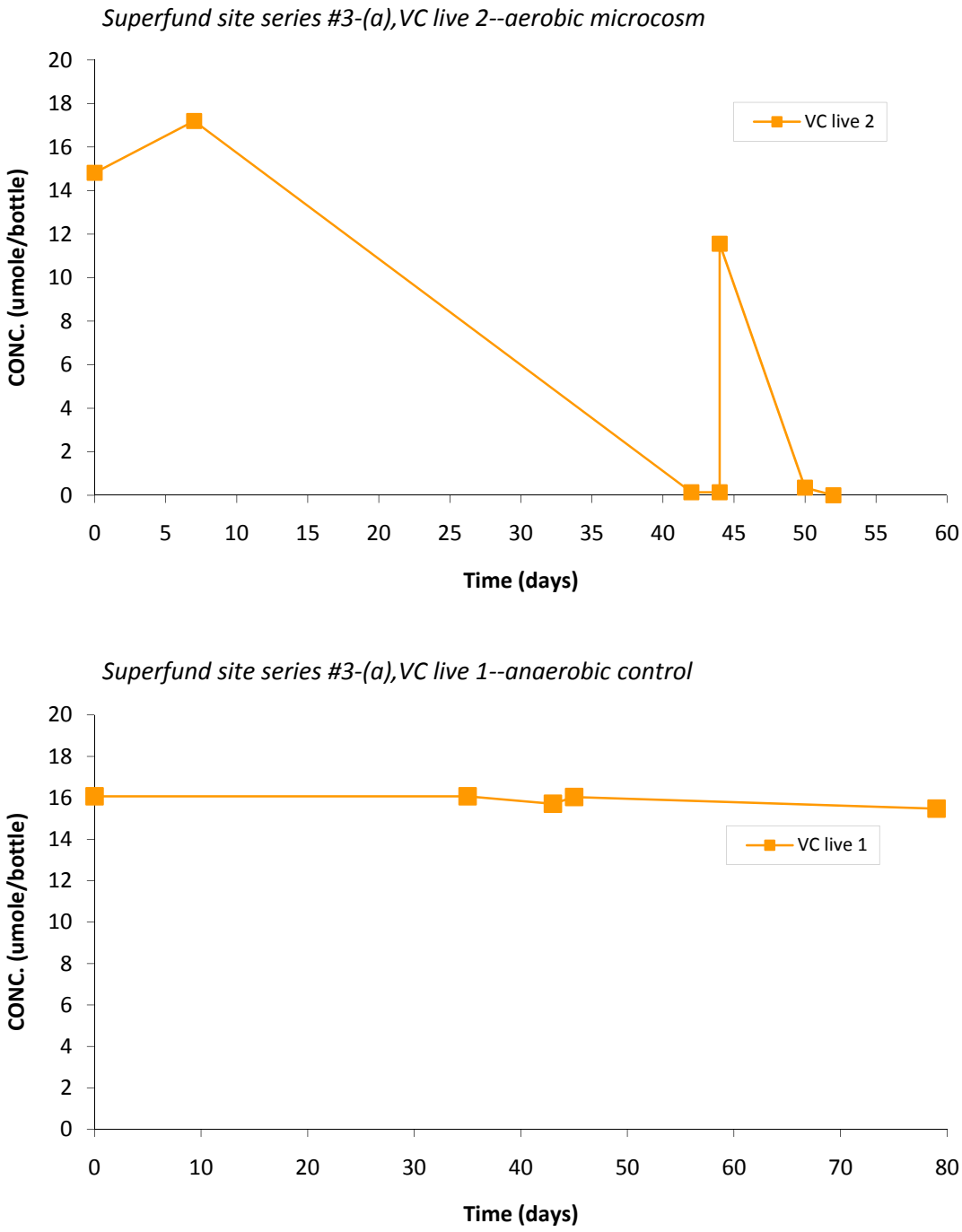
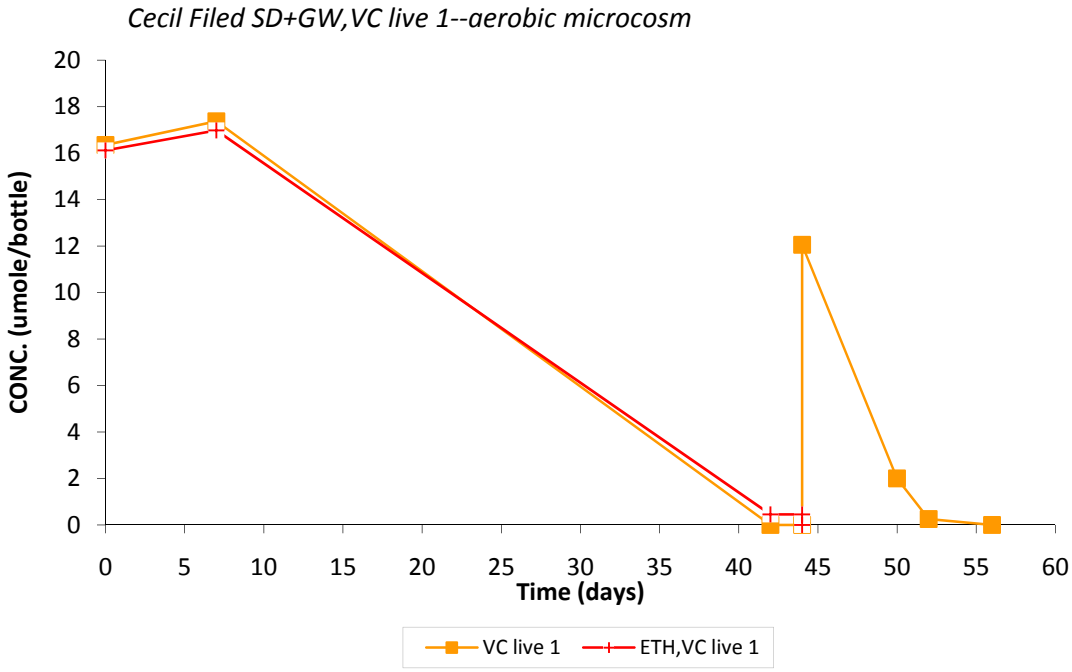


Figure 4.6.b Aerobic microcosm results for Superfund site series #3-(a).



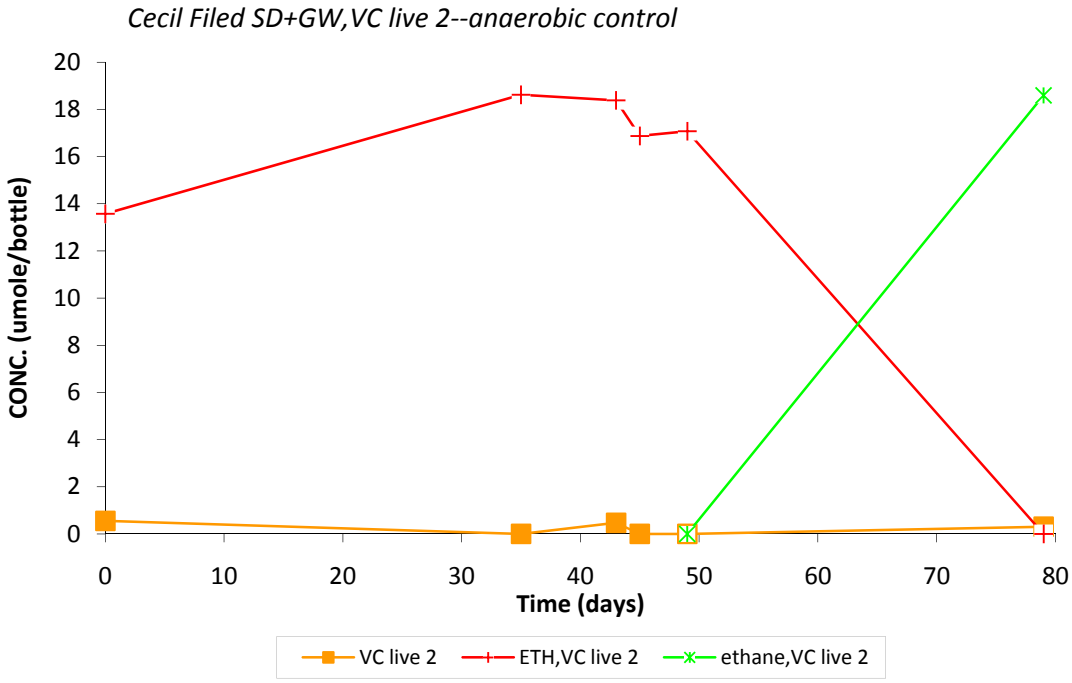


Figure 4.6.c Aerobic microcosm results for Cecil Field sediment+groundwater.

4.3. Summary of Results

In summary, of 21 series of anaerobic microcosms, 8 showed no loss of cDCE or VC (Table 4.1. Category I); 10 exhibited various extents of reductive dechlorination (usually those without Fe or Mn amendment) (Table 4.2. Category II); finally, complete reductive dechlorination occurred with Cecil Field site materials and ethane was the final product (Table 4.3. Category III).

Table 4.1 Results for Anaerobic Microcosms. Category I: No loss of cDCE/VC.

No.	Site/Source Name	Starting Date	Duration Days
1	Altus AFB, Column B2	11/19/07	158 d
4	NSB, Kings Bay KBA-11-13A	04/10/08	182 d
	NSB, Kings Bay Outcrop	04/21/08	172 d
5	NAS, Jacksonville	04/09/08	179 d
6	Unidentified Superfund Site, Series #1	06/18/08	186 d
	Unidentified Superfund Site, Series #3-(a)	06/19/08	185 d
	Unidentified Superfund Site, Series #3-(b)	07/04/08	219 d
	Unidentified Superfund Site, Series #3-(c)	07/17/08	197 d

Table 4.2 Results for Anaerobic Microcosms. Category II: Reductive Dechlorination (RD).

No.	Site/Source Name	Starting & Duration Days	Results
1	Altus AFB, Column B4	11/28/07 152 d	1 live w/o Fe (III) RD of cDCE
2	Anaerobic Sludge Supernatant, Ithaca Wastewater Treatment Plant	12/13/07 131 d	1 live w/ Fe (III) minor RD of cDCE 1 live w/o Fe(III)/Mn(IV) RD of cDCE
3	Plattsburgh AFB, Influent to the groundwater treatment plant	01/30/08 219 d	2 live w/o Fe (III) RD of VC
	Plattsburgh AFB, Idaho Avenue discharge pipe	01/31/08 173 d	2 live w/o Fe (III) RD of VC
	Plattsburgh AFB, East Flightline discharge pipe	02/01/08 172 d	1 live w/o Fe (III) RD of cDCE 1 live w/o Fe (III) minor RD of VC
6	Unidentified Superfund Site, Series #2	06/24/08 180 d	1 live w/o Fe (III) minor RD of cDCE 2 live w/o Fe (III) minor RD of VC
7	Cardinal Landfill, SO_4-1A + SO_4-2A	07/13/08 200 d	1 live w/o Fe (III) minor RD of cDCE
	Cardinal Landfill, SO_4-3A	07/15/08 199 d	All live w/ and w/o Fe(III) minor RD of cDCE
8	Aberdeen Proving Ground, WB23	08/15/08 41 d	All live w/ and w/o Fe(III) extensive RD of cDCE/VC
	Aberdeen Proving Ground, WB30	08/16/08 32 d	All live w/ and w/o Fe(III) extensive RD of cDCE/VC

Table 4.3 Results for Anaerobic microcosms.
Category III: Complete Reductive Dechlorination (RD) with ethane as the final product.

No.	Site/Source Name	Starting & Duration Days	Results
9	NAS, Cecil Field, Sediment+Groundwater	10/15/08 135 d	All live w/ and w/o Fe(III) RD of cDCE/VC Ethane in cDCE live 1; VC+Fe live 1; VC live 2
	NAS, Cecil Field, Sediment+Groundwater+Medium	10/31/08 119 d	All live w/ and w/o Fe(III) RD of cDCE/VC Ethane in all cDCE and VC live w/o Fe(III) bottles
	NAS, Cecil Field, Sediment+Medium	12/08/08 84 d	All live w/ and w/o Fe(III)/Mn(IV) RD of cDCE All live w/o Fe(III)/Mn(IV) RD of VC Ethane in one cDCE live bottle w/o Fe(III)/Mn(IV)

On the other hand, when oxygen was added to several formerly anaerobic microcosms, VC oxidation was observed in three microcosms from Undisclosed Superfund Sites Series #2 and #3-(a) and Cecil Field. No cDCE or VC oxidation occurred in other now-aerobic microcosms.

CHAPTER 5

DISCUSSION

The primary objective of this project was to develop enrichment cultures and characterize microorganisms that can oxidize cDCE and VC under Fe(III)- or Mn(IV)-reducing conditions**.** Microcosm studies were initiated using materials from 17 different sample-locations, obtained from 9 sources throughout the United States, including those where the nominally anaerobic mineralization of cDCE and/or VC was observed in previous investigations. Varieties of materials were examined to seek the potential metal-linked cDCE-/VC- oxidizers, such as aquifer sediments, streambed sediments, soil, groundwater, sludge supernatant, and column contents constructed with tree mulch, cotton gin trash, and river sand. However, after monitoring 350 or so microcosms for over one and a half years, the only observed microbial metabolic pathway in anaerobic microcosms was reductive dechlorination.

Among 10 series of microcosms that exhibited reductive dechlorination, most were microcosms devoid of Fe(III) (Table 4.2). The presence of Fe(III) also appeared to suppress methanogenesis (with the exception of Aberdeen). These results are consistent with the consensus that i) reductive dechlorination of cDCE and VC is more favorable under strongly reducing conditions, such as methanogenesis and sulfate reduction [9, 18, 27, 94, 95]; and ii) iron-reducers can generally outcompete methanogens when abundant, microbially reducible Fe(III) oxides are available [74]. On the other hand, when available organic material is abundant, it is reasonable to expect that iron-reducers would be unable to keep hydrogen levels low enough to suppress reductive dechlorination and methanogenesis.

The tendency of reductive dechlorination of chloroethenes declines as the number of chlorine substituents decreases. While dechlorination of PCE and TCE to cDCE is favored under mildly reducing conditions, i.e., NO_3^- and Fe(III) reduction, further degradation from cDCE to VC or to ethene is most likely to occur under highly reducing methanogenic or sulfate-reducing conditions [21]. Inefficient reductive dechlorination of cDCE and VC under Fe(III)-reducing conditions has been reported in other studies [21, 93]. On the other hand, mechanisms for suppression of methanogenesis under Fe(III)-reducing conditions are usually interpreted as competition between methanogens and Fe(III)-reducing bacteria for the common substrates, e.g. hydrogen and acetate [58,74]. Fe(III)-reducers have a much lower hydrogen requirement, i.e., H_2 threshold, than methanogens (0.1-0.8 nM *vs.* 5-95 nM) [54, 66]. Furthermore, besides substrate competition, a recent study also demonstrated that Fe(III)-reduction may directly affect methanogens by siphoning electrons away from methanogens during the Fe(III)-reduction process [7].

However, when electron donor availability was high, vigorous reductive dechlorination of cDCE and VC occurred in Fe(III)-amendment microcosms for Aberdeen Proving Ground, accompanied by the accumulation of Fe(II) and a large output of methane. Aberdeen sediment was very "mucky," and obviously organic-rich. Previous studies have demonstrated the significance of substrate availability on microbial interspecies competition. Lovley and Phillips, as well as other researchers, have shown that the formerly inhibited metabolic processes under more-oxidizing conditions could be overcome in the presence of excess hydrogen and acetate [50, 58, 88]. The site materials from Aberdeen Proving Ground came from wetland sediments. Fermentation of the copious organic matter in such material, therefore, could provide a steady release of hydrogen and acetate that were sufficient to sustain, simultaneously, the functions of Fe(III)-reducers, chlororespirers, and methanogens.

Similarly, reductive dechlorination occurred in all live microcosms for three series from Cecil Field, except for four VC live bottles amended with Fe(III) or Mn(IV) constructed with sediments and a basal medium. Complete reductive dechlorination from cDCE or VC to ethene, followed by further reduction of ethene to ethane under methanogenesis was observed in eight of these microcosms, one of which was amended with Fe(III). These observations agree with those from other studies that reduction from ethene to ethane is associated with strongly reducing methanogenic conditions [18, 27]. That the addition of Fe(III) did not inhibit reductive dechlorination and methanogenesis, again, was most probably a result of abundant hydrogen and acetate sources continuously produced by fermentative microbes metabolizing a range of organic matters in these rich, bed sediments.

Despite the considerable number of microcosms prepared for this study, no signs of cDCE/VC oxidation were observed, even with site materials collected from the same locations where cDCE/VC mineralization activity was previously reported by other researchers. Bradley and Chapelle first reported anaerobic mineralization of VC in Fe(III)-reducing, shallow aquifer sediments collected from NAS Cecil Field and Plattsburgh AFB in 1996 [9]. Subsequently, the same research group published a series of investigations on DCE/VC anaerobic mineralization under differing electron-accepting processes, most of which were based on microcosm studies using creek bed sediments collected from Cecil Field — from the same location where we obtained material in our study. The previous studies included microbial mineralization of DCE and VC under Fe(III)-reducing, SO_4^{2-}-reducing and methanogenic conditions [10, 12, 14], DCE/VC anaerobic oxidation under humic acid-reducing conditions [11], and acetogenic microbial oxidation of VC [15, 16]. Likewise, we also obtained groundwater and soil samples, courtesy of Dr. David Freedman (Clemson University), where VC mineralization was observed several years ago (although the activity was

later lost for unknown reasons). However, given the elapse of time between these previous studies and ours, the more recent collection of materials from the same locations as used in previous studies would not guarantee similar results.

Temporal and spatial variations of terminal electron-accepting processes can exert a strong influence on the pathways and rates of cDCE/VC degradation. Field and laboratory studies on biotransformation of petroleum hydrocarbon contaminants have demonstrated that terminal electron-accepting processes (TEAP) can shift from one to another in response to natural or anthropogenic changes in groundwater recharge, discharge, and withdrawal, which would significantly alter the pathways and products of organic-contaminant biodegradation [12, 21, 88].

At NAS Cecil Field, substantial temporal variability of the creek bed sediments, even during a short time frame, was shown in Bradley and Chapelle's studies a decade ago. Microbial activity varied over time from predominantly oxidized products to entirely reductive dechlorination products [Bradley, personal communication]. Apart from CO_2, ethene and ethane were detected as the concurrent end products of VC biodegradation under methanogenic conditions [15, 17]. Another annotation on temporal variability is that the activity of anaerobic DCE/VC oxidation under humic acid reduction was never dependably produced again by the same research team [Bradley, personal communication]. In addition, a contaminant plume is also dynamic in spatial distribution. For example, the groundwater and soil samples obtained from Dr. Freedman were collected further downgradient from the location his lab used for the original study several years ago, in that the highly reducing zone of the plume has since moved downgradient [Freedman, personal communication]. Freedman's lab was conducting similar microcosm studies on anaerobic VC mineralization concurrently with ours, but using different experimental methods. They tested VC degradation activity under various electron acceptors, including amorphous Fe(III), EDTA-Fe(III),

Mn(IV), sulfate and anthraquinone-2,6-disulfonate (AQDS) [49]. However, anaerobic oxidation of VC was neither observed in their microcosm study nor ours. In our study, only minor reductive dechlorination occurred in three of the live microcosms for these sites; others exhibited no loss of cDCE or VC.

The failure to observe anaerobic cDCE/VC oxidation can also be attributable to an integration of other variables. For example, method or personnel changes in sample collection, experimental setup, analysis procedures and even flawed monitoring can introduce variability into experimental results. As a consequence, it can be a hit-or-miss event, in terms of finding just the right conditions for microbial anaerobic mineralization.

Although the results of our study, as well as those from other studies, illustrate the difficulty of finding metal-linked cDCE/VC- oxidizing microorganisms, a question has been raised in the meantime: is the previously observed "anaerobic mineralization" really, in all cases, anaerobic?

Compared with strongly reducing electron-accepting processes where oxygen is strictly absent (e.g., methanogenesis), iron reduction and manganese reduction are relatively oxidized "anaerobic" processes where oxygen is not detectable but existent [19]. Furthermore, Coleman *et al.* have reported that effective aerobic oxidation of VC can occur under extremely low oxygen concentrations. The measured half-velocity constants (Ks) for five strains of VC-assimilating bacteria are from 0.03 to 0.3 mg/liter; their minimum oxygen requirements (O_2 thresholds) are as low as 0.02 to 0.1 mg/L [25]. Thus, trace amounts of O_2 existing in ostensibly O_2-free systems might potentially contribute to mineralization of the lesser-chlorinated compounds.

Experimental bias resulting from oxygen contamination is thus another concern in judging the authenticity of the formerly found "anaerobic mineralization." As mentioned previously (Materials and Methods), Bradley and Chapelle have discovered,

in their recent studies, that small amounts of air were accidentally introduced to the nominally anoxic microcosms and reactors during sampling procedures, which is a common risk in anaerobic experiments. Moreover, unexpectedly, a strain of aerobic VC-oxidizing bacteria, a *Mycobacterium,* was isolated from Freedman's "anaerobic" enrichment culture. Given the fact that only a small proportion of their previous microcosms showed positive activity (2/15 and 1/18), which was eventually lost [49], oxygen contamination might be one of the possible explanations for previously observed mineralization (though it must be pointed out that Freedman's group did seem to link disappearance of VC to appearance of acetate).

Finally, a recent investigation on biotransformation of cDCE and VC in Fe(III)-reducing cultures developed from landfill leachate sediment demonstrated that the enrichment culture was capable of degrading cDCE and VC without accumulation of reductive dechlorination products [43]. However, this oxidative activity was proved not to be associated with Fe(III)-reduction, SO_4^{2-}-reduction, or methanogenesis, but rather associated with other unclear microbial mechanisms.

A comprehensive consideration of the facts mentioned above gave rise to the hypothesis that what has appeared to be anoxic mineralization of cDCE and VC in some microcosm studies might in fact have been aerobic mineralization under extremely low O_2 concentrations. To initiate an investigation on this possibility, 10 mL of pure O_2 was added to 16 of the previously anaerobic microcosms to assess if aerobic cDCE-/VC-oxidizers could survive after being incubated anaerobically, and, if so, to ascertain the speed of their resurgence.

After a 44-day incubation, VC-oxidizing activity was exhibited in three of the eight VC-amended experimental microcosms — Superfund sites series #2 and # 3-(a) and NAS Cecil Field. Repeat additions of VC were completely consumed within 6-12 days. No oxidation of cDCE was observed in any of the eight cDCE-amended

microcosms. Instead, robust reductive dechlorination occurred in Cecil Field microcosm, most likely because the added O_2 was depleted by various biotic or abiotic mechanisms in the organic-rich material in the microcosm, and reductive dechlorination could again become the predominant metabolic process.

Results support the notion that aerobic VC-oxidizers can persist even after extended anaerobic incubation. What remains is to see if aerobic VC oxidation can occur under the influence of a low, steady influx of O_2 that enables only extremely low oxygen concentrations to be maintained.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Anaerobic oxidation of cDCE and VC under Fe(III)- and Mn(IV)-reducing conditions metabolized by indigenous microorganisms at chloroethene-contaminated sites has been hypothesized as a significant alternative metabolic pathway to reductive dechlorination [9, 23]. However, anaerobic mineralization is still an area of active research since microorganisms capable of oxidative cDCE/VC degradation have not been identified, and neither have the metal-linked metabolic pathways been clearly elucidated [18]. Aiming at the development of enrichment cultures exhibiting cDCE/VC mineralization activity under iron- or manganese-reducing conditions, we hereby initiated this microcosm study. Over a one-and-one-half-year period, 21 series of microcosms – about 350 microcosms in total – were constructed, with materials from 17 different sampling locations from 9 sources in the USA. The change of chloroethenes, along with the accumulation of Fe(II) and Mn(II), was monitored periodically for each series of microcosms during an approximately 6 month of incubation period.

The following results were achieved:

(1) cDCE or VC mineralization under Fe(III)- or Mn(IV)-reducing conditions was not discovered in any of the 350 microcosms. Instead, reductive dechlorination was the only anaerobic metabolic pathway observed in this study: 8 series of microcosms showed no loss of cDCE or VC; 10 exhibited various extents (from minor to complete) of reductive dechlorination; complete reductive dechlorination occurred with Cecil Field site materials and ethane was the final product in 8 Cecil Field microcosms.

(2) Most microcosms showing reductive dechlorination were those unamended with Fe(III) or Mn(IV) and without methane production. This is consistent with the results from previous studies that addition of Fe(III) or Mn(IV) can suppress reductive dechlorination and methanogenesis.

(3) When electron donor was not a limiting factor, extensive reductive dechlorination with large methane output was observed in Fe- and Mn-amended microcosms constructed with materials from Aberdeen Proving Ground and NAS Cecil Field. Site materials from these two sites are characterized as organic-rich, likely resulting in abundant hydrogen and acetate sources to satisfy the metabolic needs for Fe(III)-/Mn(IV)-reducers, reductive dechlorinators, and methanogens.

(4) In view of the considerable, unsuccessful efforts made to characterize anaerobic cDCE/VC-oxidizers (in studies by both our lab and other research teams), we perceive considerable challenges to the identification of anaerobic cDCE/VC oxidizing microorganisms. Temporal and spatial variations as well as other variables, such as method or personnel changes in sample collection, different methods in experimental setup, and flawed monitoring could perhaps partially explain the lack of success that we and others have encountered in attempting to reproduce the previously observed cDCE/VC mineralizations by other researchers. It appears that anaerobic cDCE/VC oxidation – if it exists at all – is not a microbial metabolic process as widespread as reductive dechlorination. However, considering the decade-long effort before the final isolation of *D. ethenogenese* 195, characterization of metal-linked cDCE/VC oxidizers, if they really exist, might be a similarly lengthy process – or even more so. The metal-reducing environment – existing as somewhat of a transition zone between truly

aerobic and highly reductive, anaerobic zones of a contaminant plume – is a comparatively smaller niche than either of those that flank it. Given that zone boundaries shift with time, this is a situation that makes locating anaerobic oxidizers all the more difficult.

(5) While not denying the existence of anaerobic cDCE/VC oxidizing microorganisms, we propose a hypothesis (based on previous research results and personal communication with other research teams) that what has been reported to be anaerobic oxidation (at least in some studies) might have in fact been aerobic oxidation sustained under extremely low fluxes of oxygen. We added 10 mL of pure oxygen to 16 of cDCE- and VC-fed microcosms that were previously operated anaerobically and obtained three active cultures showed VC-oxidizing activity (Superfund sites series #2 and #3-(a) and Cecil Field). Future work is needed to continue the investigation on aerobic VC oxidation under low O_2 concentration.

APPENDEX I: SUMMARY OF MICROCOSMS

Table I-1 Summary of Microcosms.

Site No.	Site Name	Microcosm Inoculum	Treatments					
			Fe (III) reducing		unamended		Mn (IV) reducing	
			cDCE	VC	cDCE	VC	cDCE	VC
1	Altus Air Force Base	Column B2 effluent	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Column B4 effluent	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
2	Ithaca Wastewater Treatment Plant	Sludge supernatant	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed
3	Plattsburgh Air Force Base	Influent to groundwater treatment system	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Groundwater from Idaho Ave Discharge Pipe	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Groundwater from East Flightline Discharge Pipe	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
4	Naval Submarine Base, Kings Bay	Sediment from KBA-11-13A	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Sediment from KB Outcrop	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
5	Naval Air Station Jacksonville	Sediment	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
6	Undisclosed Superfund Sites	Series #1: soil and groundwater	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Series #2: soil + groundwater + medium	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Series #3-(a): soil + groundwater	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Series #3-(b): soil+ groundwater + medium	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		

Table I-1 Continued

Site No.	Site Name	Microcosm Inoculum	Treatments					
			Fe (III) reducing		unamended		Mn (IV) reducing	
			cDCE	VC	cDCE	VC	cDCE	VC
6	Undisclosed Superfund Sites	Series #3-(c): soil+ groundwater + medium	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
7	Cardinal Landfill	SO_4-1A + SO_4-2A	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		SO_4-3A	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
8	Aberdeen Proving Ground	Groundwater and sediments from WB 23	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Groundwater and sediments from WB 30	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
9	Naval Air Station Cecil Field	Sediment+ Ground water	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Sediment+Ground water+Medium	2 active 2 killed	2 active 2 killed	2 active 2 killed	2 active 2 killed		
		Ground water+ Medium	2 active 2 killed	2 active 2 killed	2 active 1 killed	2 active 1 killed	2 active 2 killed	2 active 2 killed

APPENDIX II: CONTENTS OF MICROCOSMS

Table II-1A Site/Source 1: Altus AFB, Column B2.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/DS water	live/killed	
B2 Effluent	1.5 mL	94 mL (yeast extract 0.05 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-1B Site/Source 1: Altus AFB, Column B4.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe	live/killed	
B4 Effluent	1.5 mL	94 mL (yeast extract 0.05 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-2 Site/Source 2: Ithaca Area Wastewater Treatment Plant.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Sludge supernatant	10 mL	85 mL (yeast extract 0.05 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL Mn(IV) stock slurry		2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL Mn(IV) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL Mn(IV) stock slurry		2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Mn(IV) stock slurry		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						24

Table II-3A Site/Source 3: Plattsburgh AFB, Influent to the groundwater treatment plant.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Groundwater	10 mL	85 mL (yeast extract 0.025 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-3B Site/Source 3: Plattsburgh AFB, Idaho Avenue discharge pipe.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Groundwater	10 mL	85 mL (yeast extract 0.025 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-3C Site/Source 3: Plattsburgh AFB, East Flightline discharge pipe.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Groundwater	10 mL	85 mL (yeast extract 0.025 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-4A Site/Source 4: NSB, Kings Bay KBA-11-13A.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Sediment	9 g	95 mL (yeast extract 0.01 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-4B Site/Source 4: NSB, Kings Bay Outcrop.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Shallow Aquifer Sediment	5 mL	95 mL (yeast extract 0.01 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-5 Site/Source 5: NAS, Jacksonville.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Freshwater Sediment	5 mL	95 mL (yeast extract 0.01 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-6A Site/Source 6: Unidentified Superfund Site, Subset #1.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Groundwater & two types of soil	90 mL groundwater; 5 g soil from MW-2113-48 #2151; 5g soil from MW-2113-48 #2119	no	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-6B Site/Source 6: Unidentified Superfund Site, Subset #2.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Groundwater & one type of soil	90 mL mixture of groundwater and medium (10:8); 7 g soil	90 mL mixture of ground water and medium (10:8)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-6C Site/Source 6: Unidentified Superfund Site, Subset #3-(a).

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Groundwater & two types of soil	90 mL groundwater; 5 g soil from each type of cores	no	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-6D Site/Source 6: Unidentified Superfund Site, Subset #3-(b).

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Groundwater & two types of soil	90 mL groundwater; 5 g soil from each type of cores	90 mL mixture of ground water and medium (17:1)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-6E Site/Source 6: Unidentified Superfund Site, Subset #3-(c).

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Groundwater & two types of soil	90 mL groundwater; 5 g soil from each type of cores	90 mL mixture of ground water and medium (17:1)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-7A Site/Source 7: Cardinal Landfill, SO_4-1A + SO_4-2A.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Supernatant from old microcosms	90 mL mixture of the supernatant (7% v/v), groundwater from Superfund series #3 (5%, v/v) and medium (88%, v/v)	88% (v/v) medium (yeast extract 0.01 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-7B Site/Source 7: Cardinal Landfill, SO_4-3A.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Supernatant form old microcosms	90 mL mixture of the supernatant (3% v/v), and medium (97%, v/v)	97% (v/v) medium (yeast extract 0.01 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-8A Site/Source 8: Aberdeen Proving Ground, WB23.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Freshwater tidal wetland sediments	25 g	77.5 mL (yeast extract 0.01 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-8B Site/Source 8: Aberdeen Proving Ground, WB30.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Freshwater tidal wetland sediments	25 g	77.5 mL (yeast extract 0.01 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-9A Site/Source 9: NAS, Cecil Field, Sediment+Groundwater.

Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Streambed sediment+ Groundwater	25 g mixed sediment from 3 locations ; 90 mL ground water	No	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

Table II-9B Site/Source 9: NAS, Cecil Field, Sediment+Groundwater+Medium.

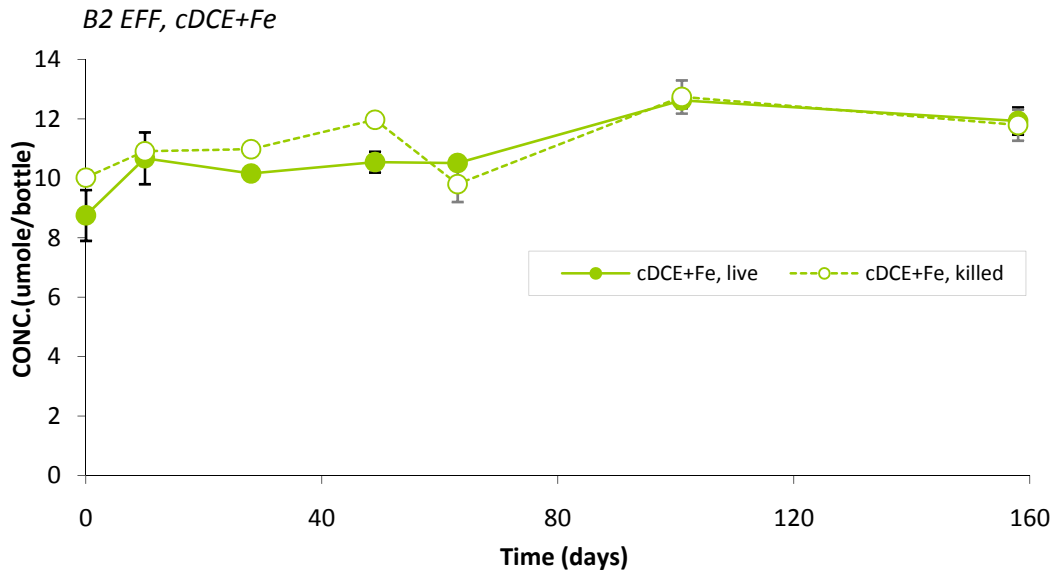
Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Streambed sediment+ Groundwater +Medium	25 g mixed sediment from 3 locations; 90 mL mixture of ground water and medium (1:1)	90 mL mixture of ground water and medium (1:1) (yeast extract 0.01 g/L)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		2
Total						16

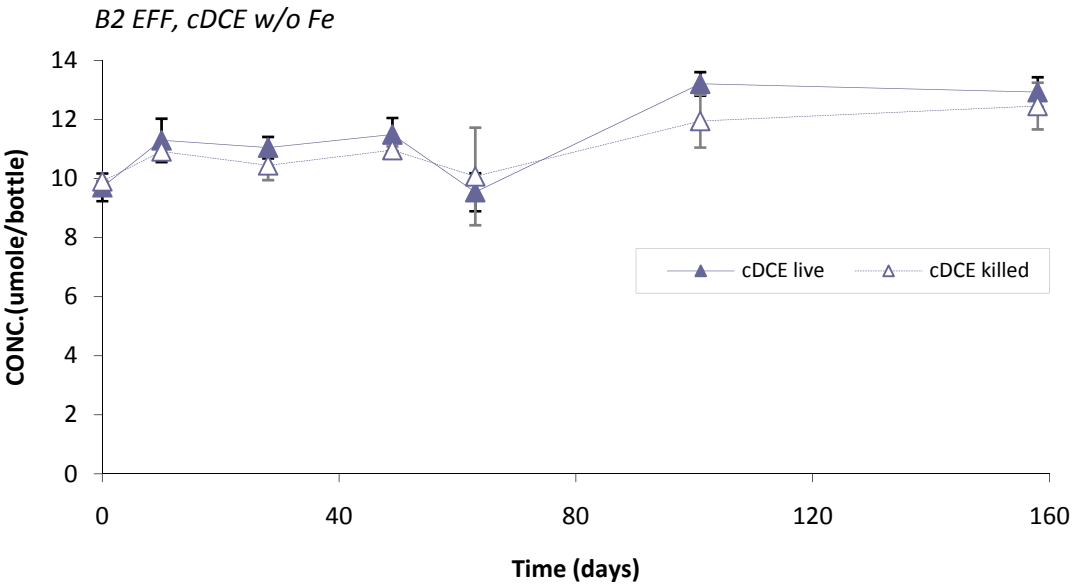
Table II-9C Site/Source 9: NAS, Cecil Field, Sediment+Medium.

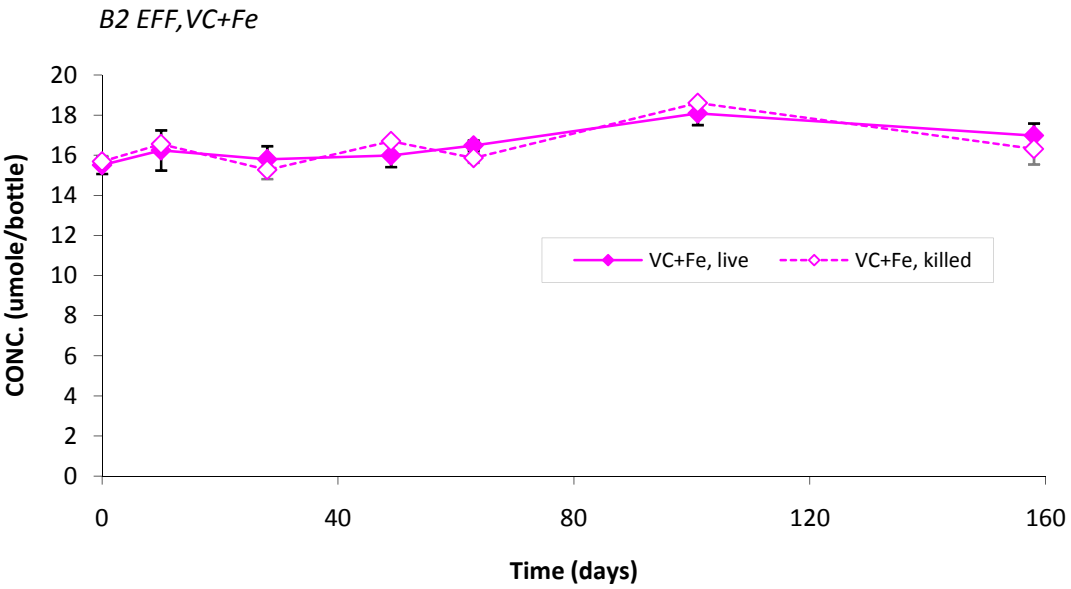
Inoculum type	Conditions					No. of bottles
	Inoculum	Medium	cDCE/VC	Fe/Mn	live/killed	
Streambed sediment+ Medium	25 g mixed sediment from 3 locations	85 mL (no yeast extract)	150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	live	2
			150 µL cDCE saturated solution	5 mL Mn(IV) stock slurry		2
			150 µL cDCE saturated solution	5 mL DS water		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL Mn(IV) stock slurry		2
			400 µL neat VC	5 mL DS water		2
			150 µL cDCE saturated solution	5 mL Fe(III) stock slurry	killed	2
			150 µL cDCE saturated solution	5 mL Mn(IV) stock slurry		2
			150 µL cDCE saturated solution	5 mL DS water		1
			400 µL neat VC	5 mL Mn(IV) stock slurry		2
			400 µL neat VC	5 mL Fe(III) stock slurry		2
			400 µL neat VC	5 mL DS water		1
Total						22

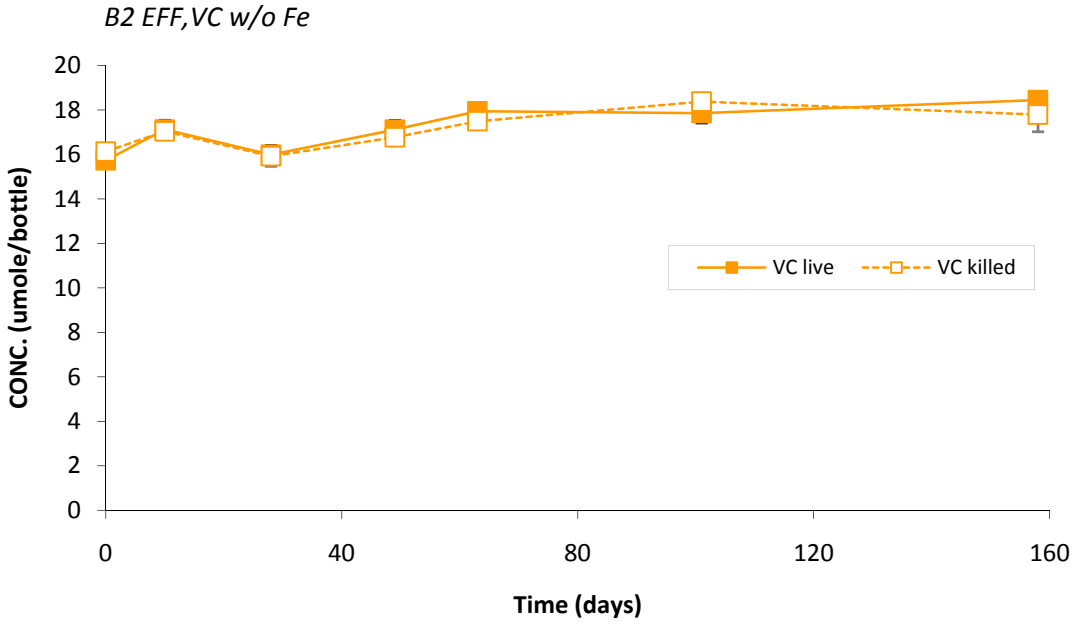
APPENDIX III: RESULTS FOR ANAEROBIC MICROCOSMS

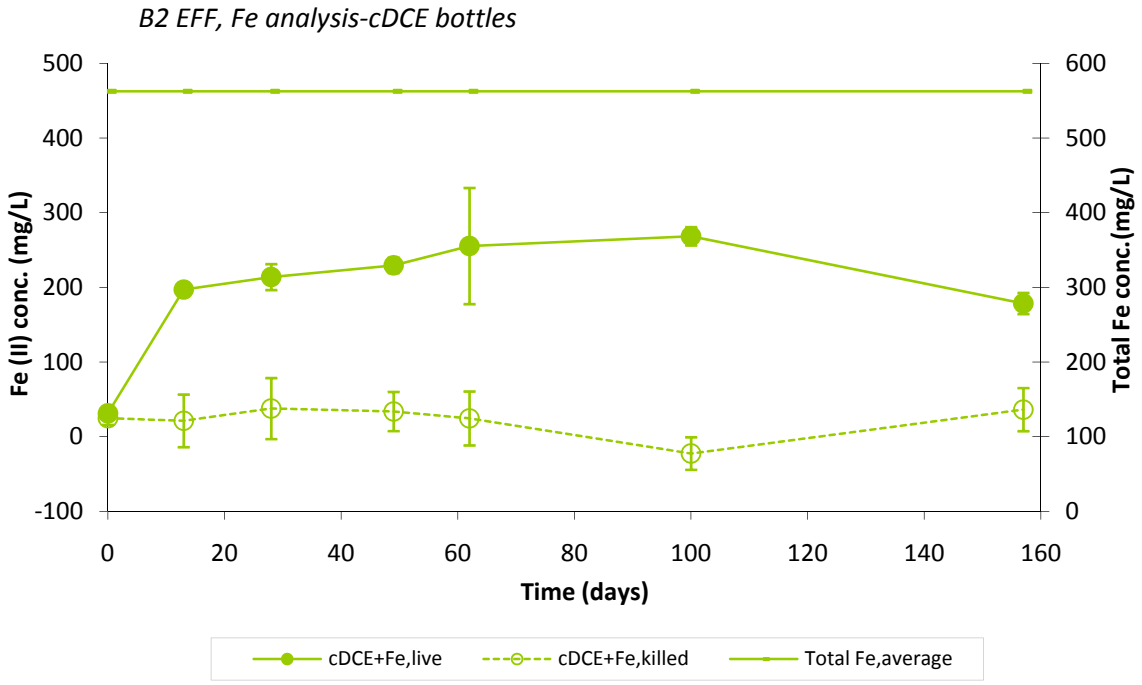
1-A. Site/Source 1: Altus AFB, Column B2:

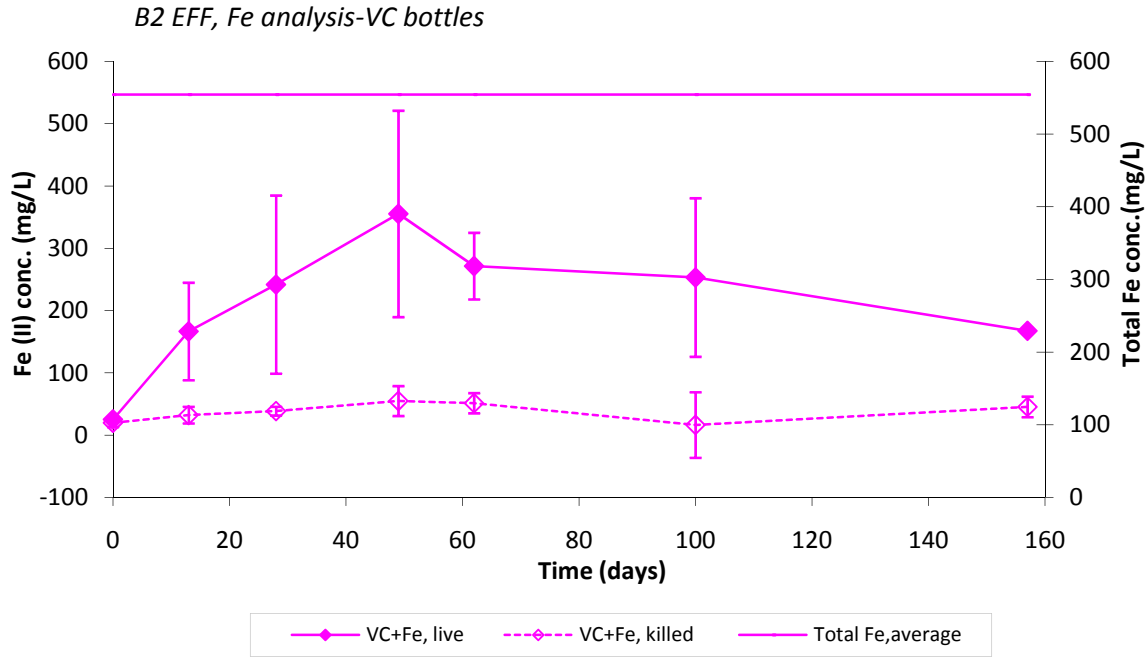




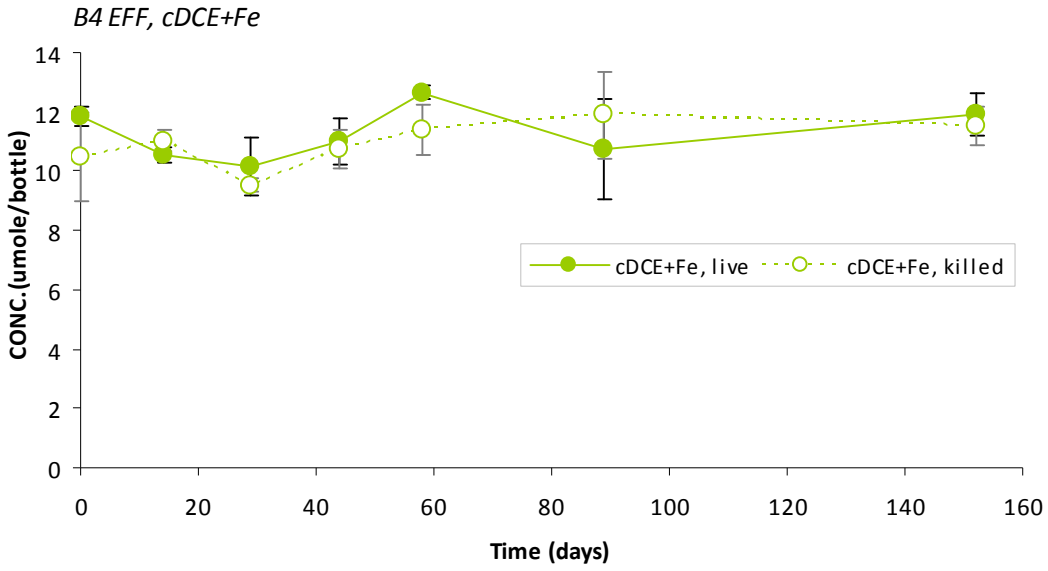

B2 EFF,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
2
4
6
8
10
12
14
16
18
20
0
40
80
120
160

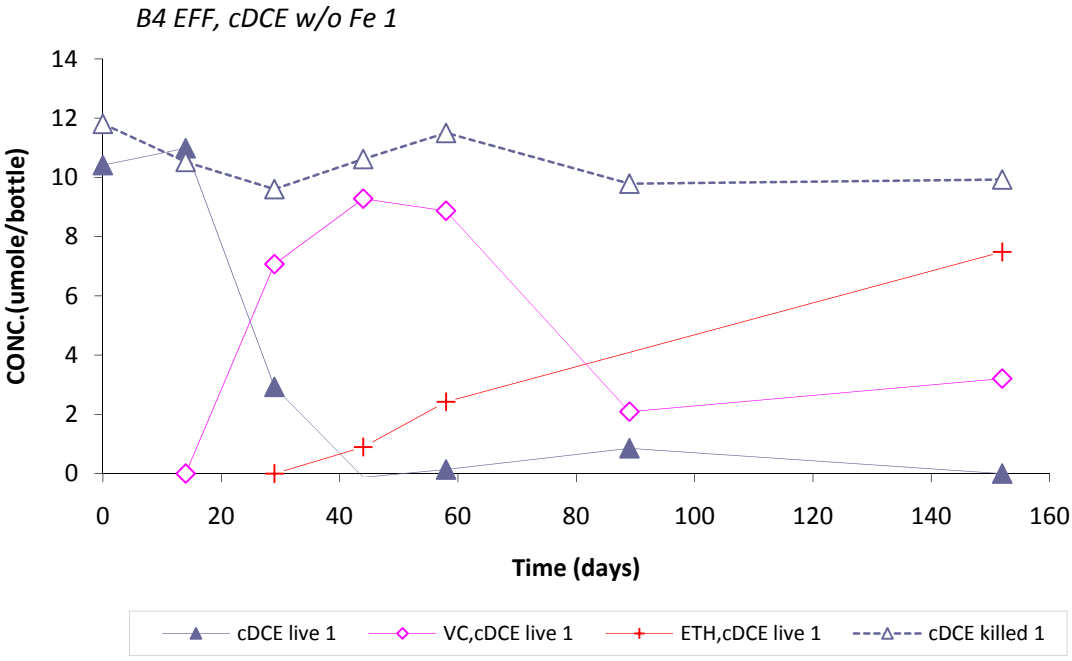

B2 EFF,VC w/o Fe
CONC. (umole/bottle)
Time (days)
VC live
VC killed
0
2
4
6
8
10
12
14
16
18
20
0
40
80
120
160

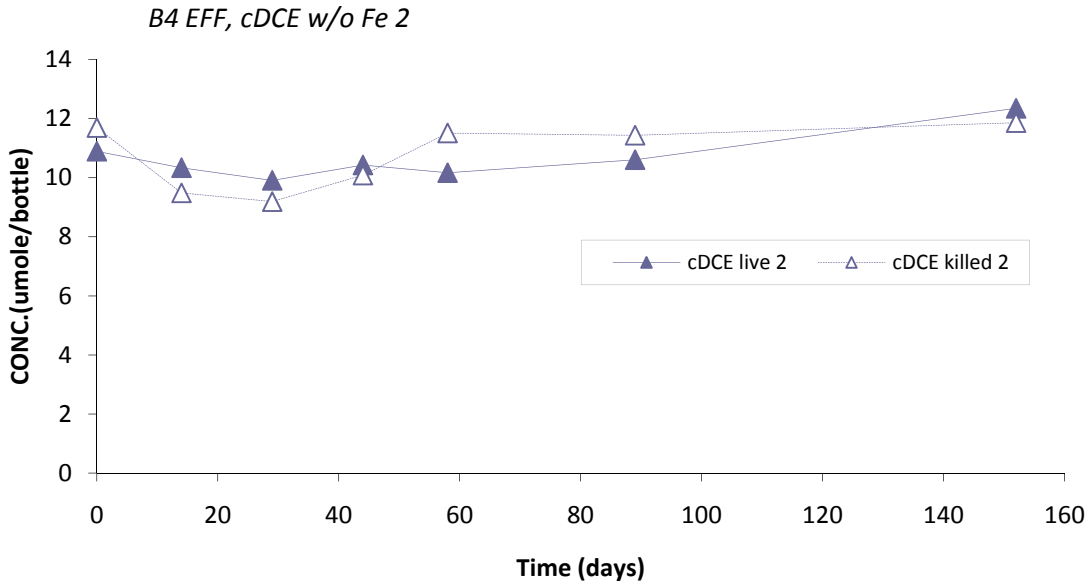

B2 EFF, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

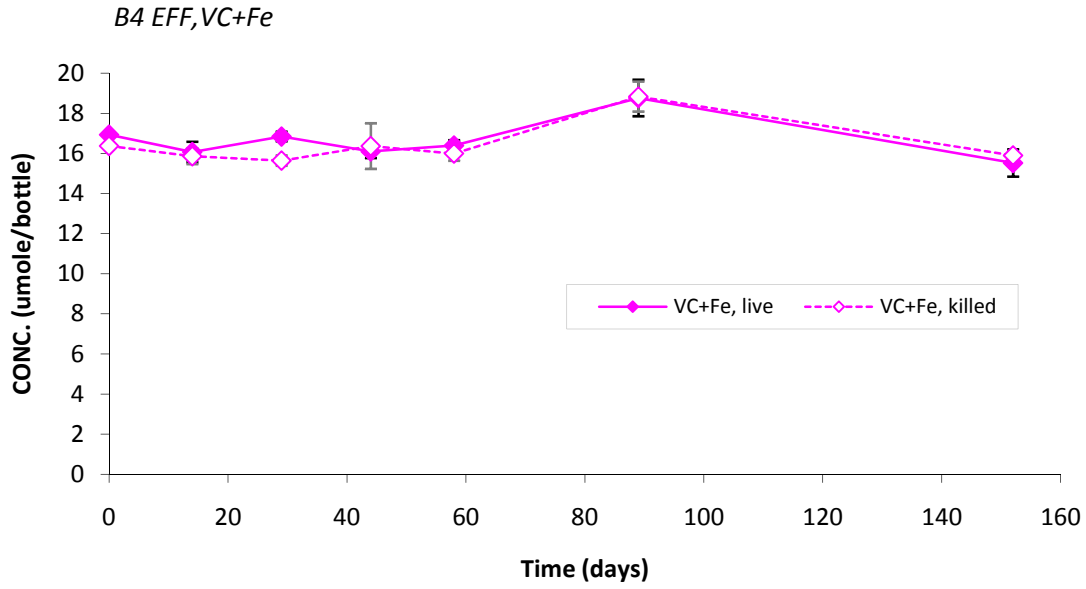

B2 EFF, Fe analysis-VC bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
VC+Fe, live
VC+Fe, killed
Total Fe,average

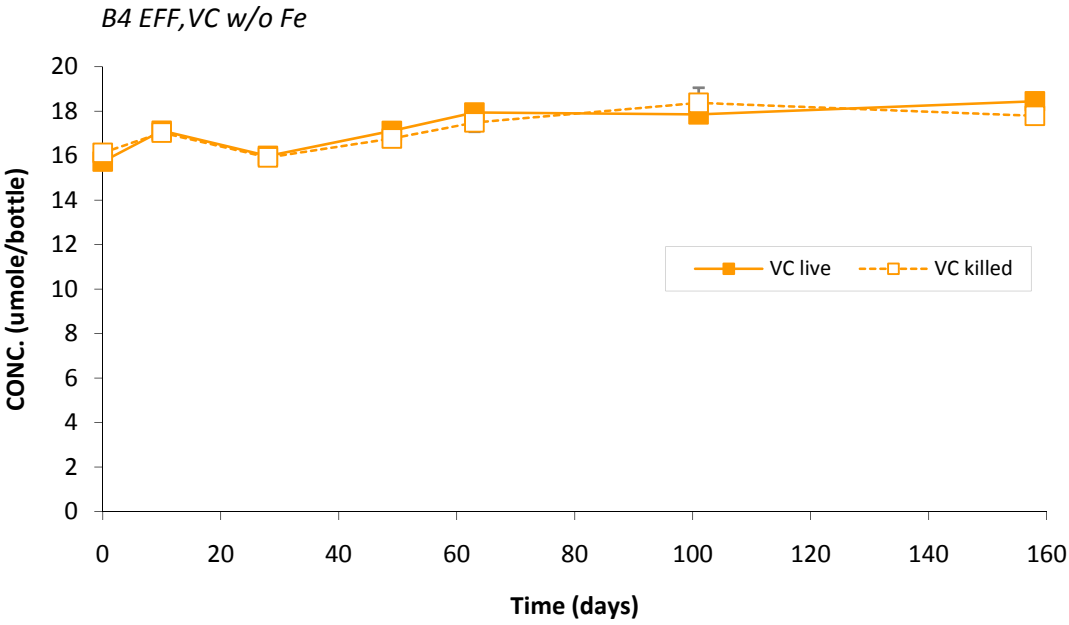
1-B. Site/Source 1: Altus AFB, Column B4:

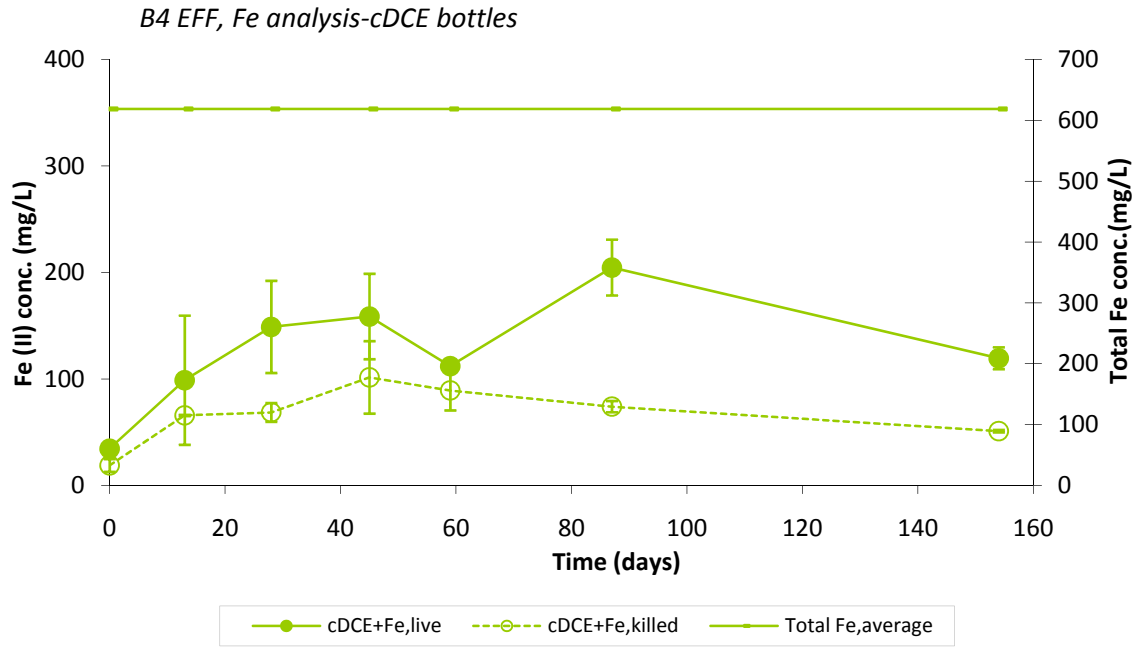


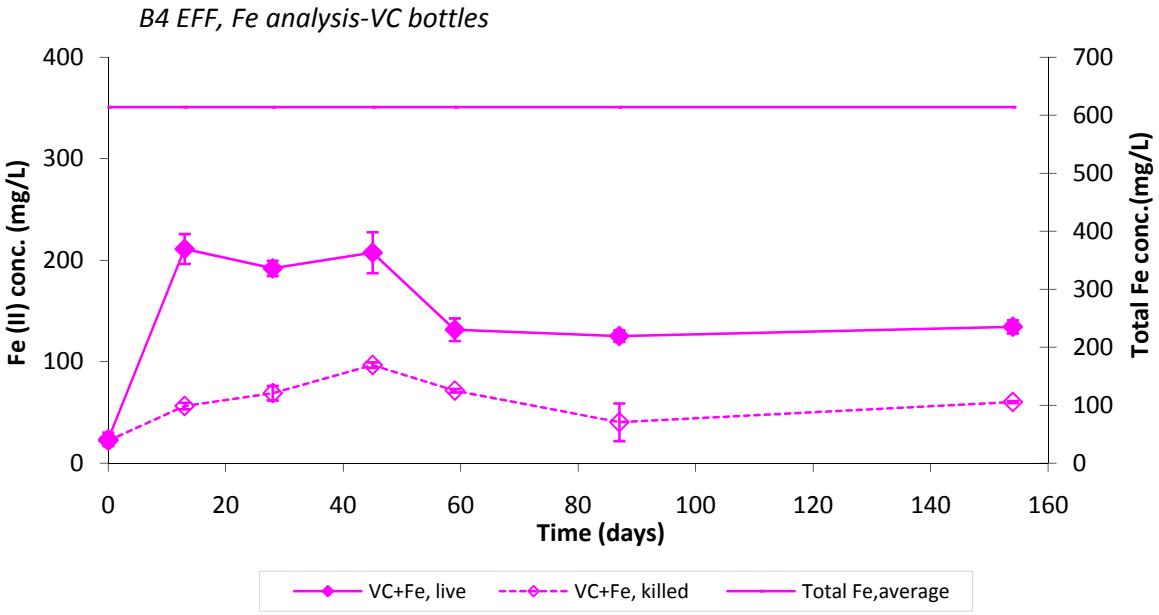



B4 EFF, cDCE w/o Fe 2
CONC.(umole/bottle)
Time (days)
cDCE live 2
cDCE killed 2

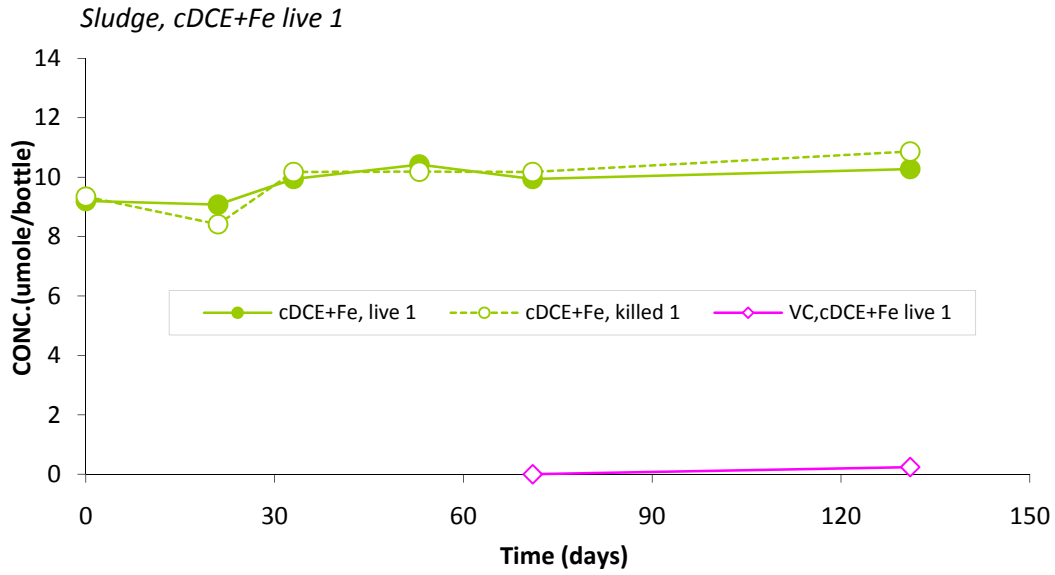

B4 EFF,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed

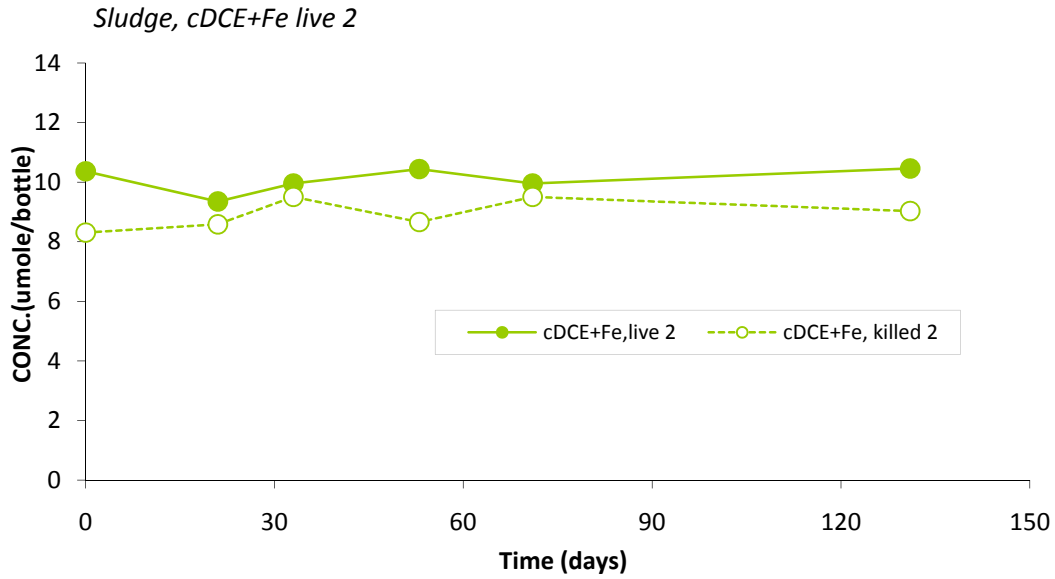

B4 EFF,VC w/o Fe
CONC. (umole/bottle)
Time (days)
VC live
VC killed

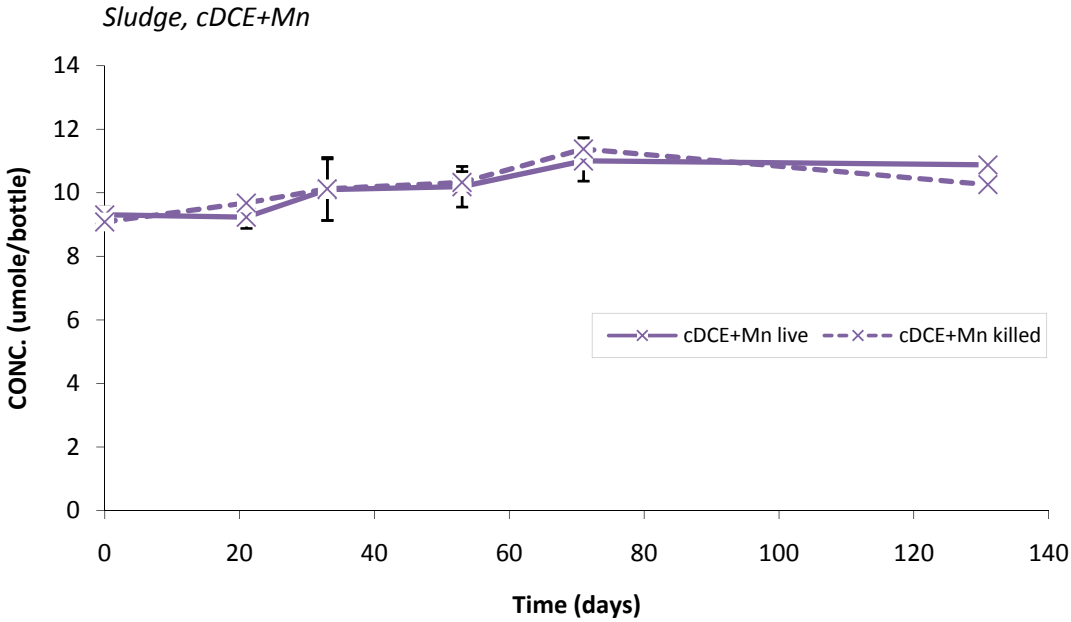

B4 EFF, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

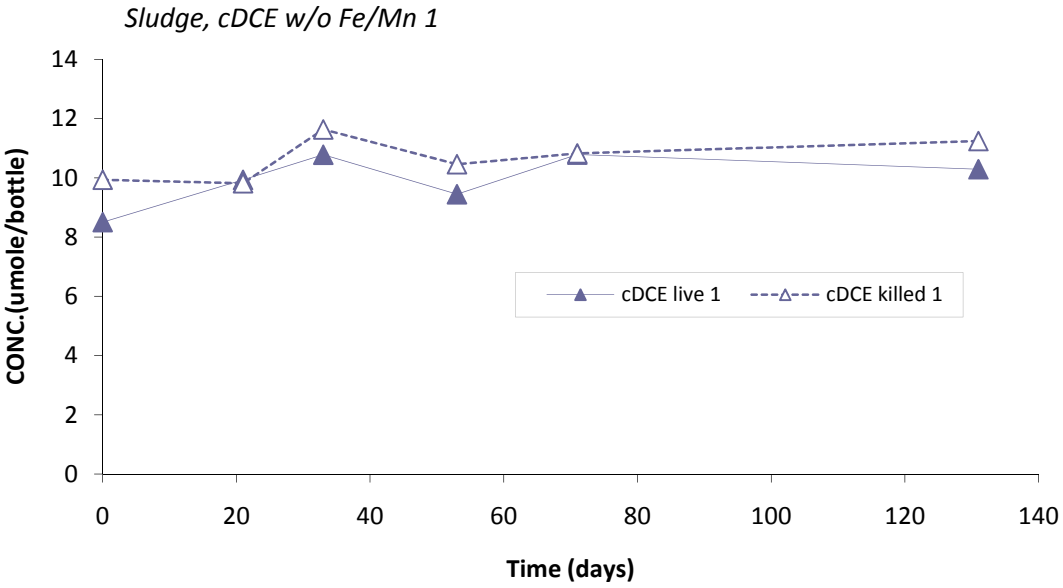


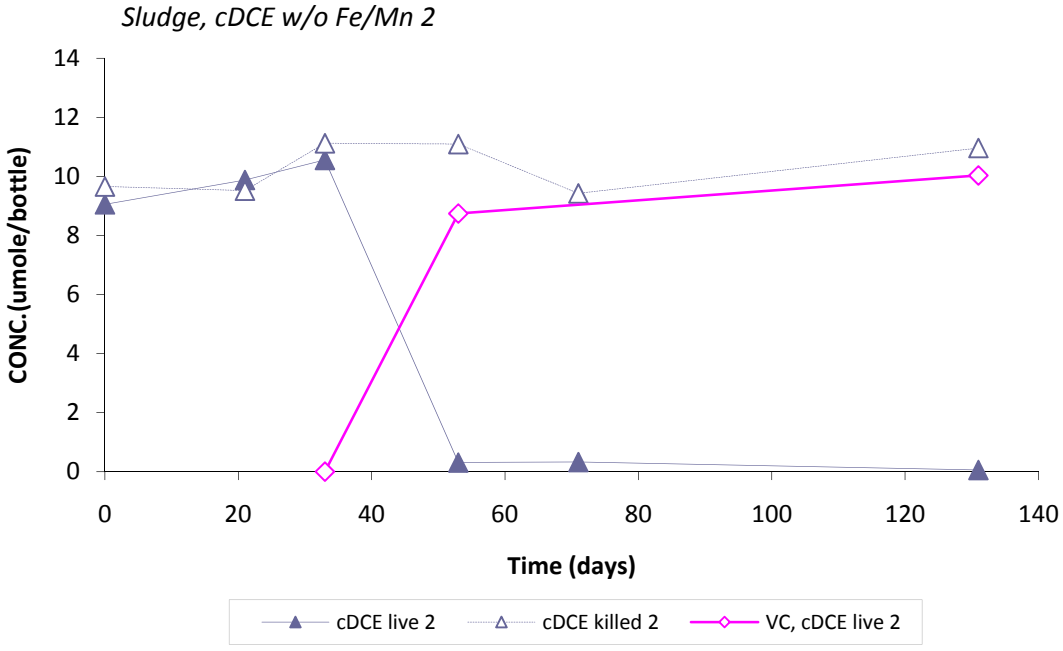
2-A. Site/Source 2: Ithaca Area Wastewater Treatment Plant:

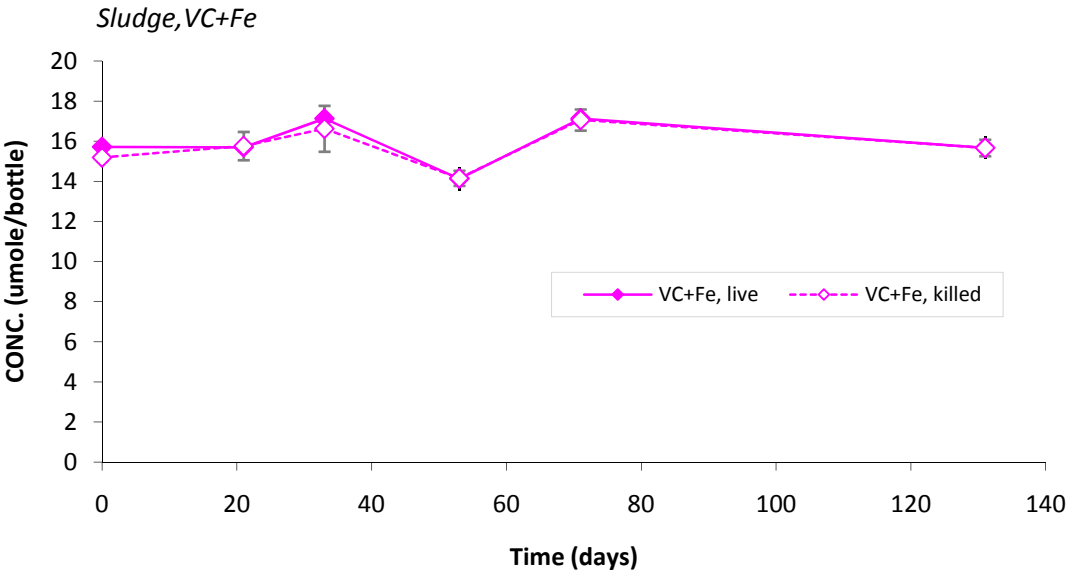


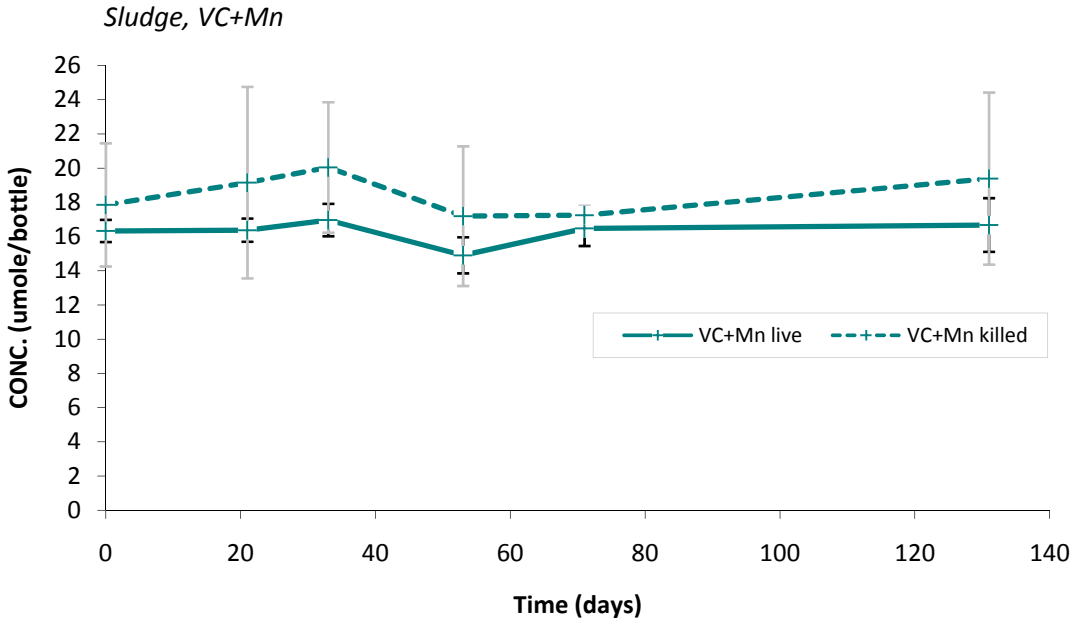

Sludge, cDCE+Fe live 2
CONC.(umole/bottle)
Time (days)
cDCE+Fe,live 2
cDCE+Fe, killed 2

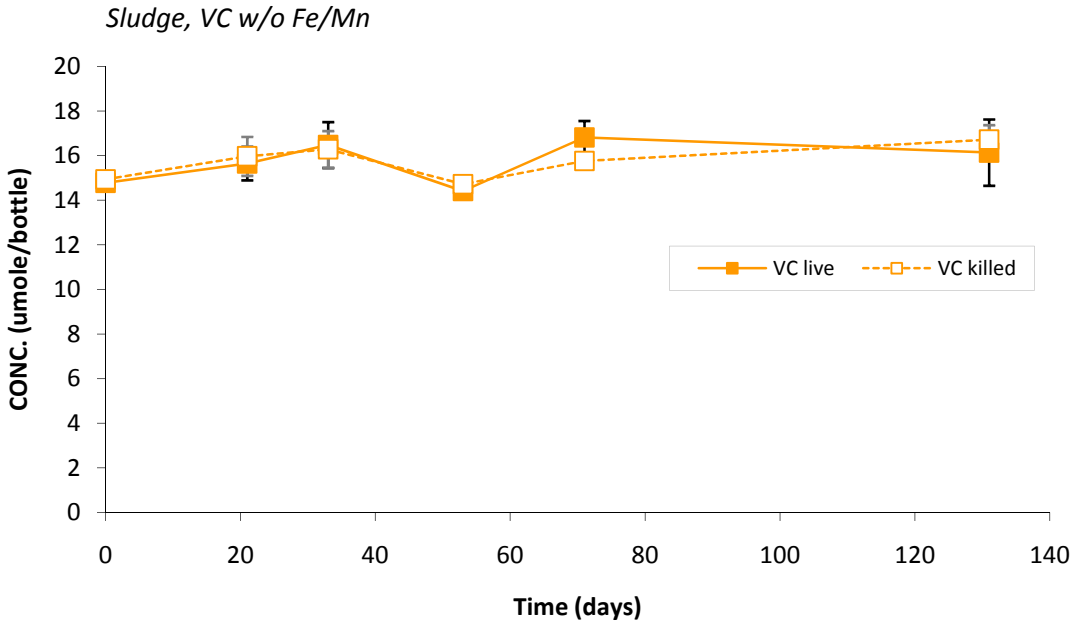

Sludge, cDCE+Mn
CONC. (umole/bottle)
Time (days)
cDCE+Mn live
cDCE+Mn killed

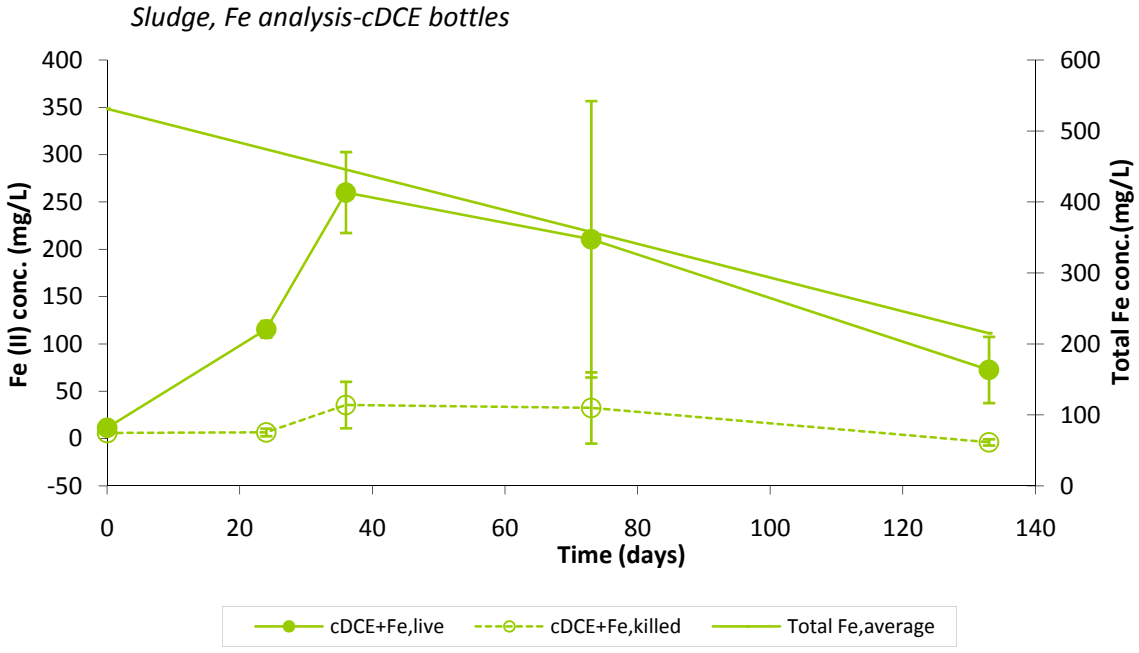

Sludge, cDCE w/o Fe/Mn 1
CONC.(umole/bottle)
Time (days)
cDCE live 1
cDCE killed 1

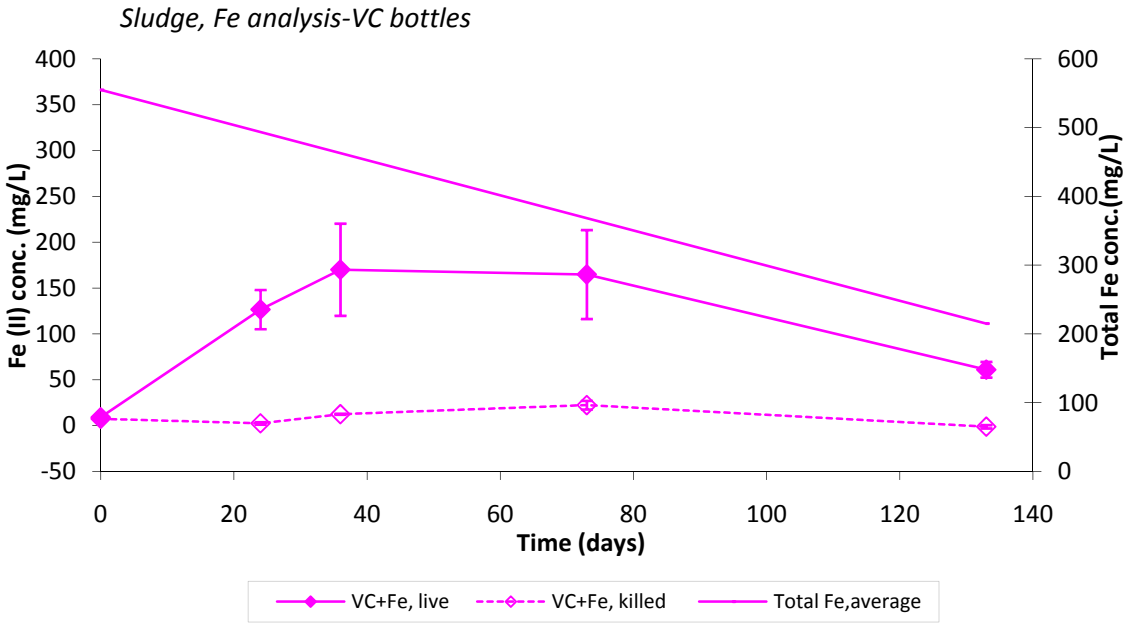

Sludge, cDCE w/o Fe/Mn 2
CONC.(umole/bottle)
Time (days)
cDCE live 2
cDCE killed 2
VC, cDCE live 2

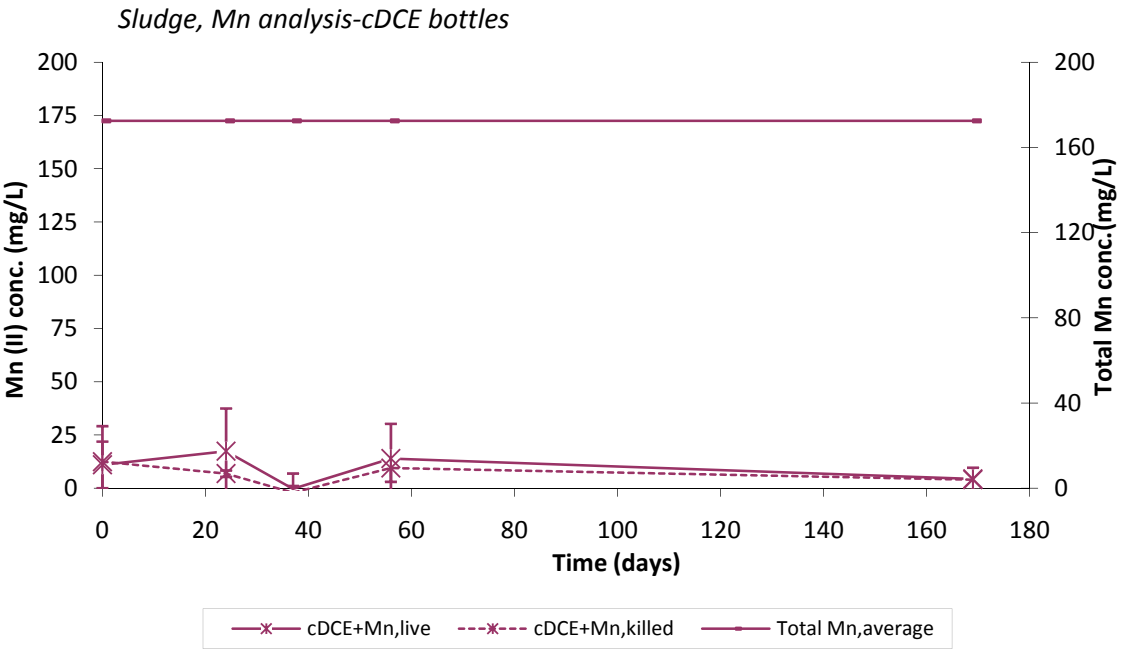

Sludge,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed

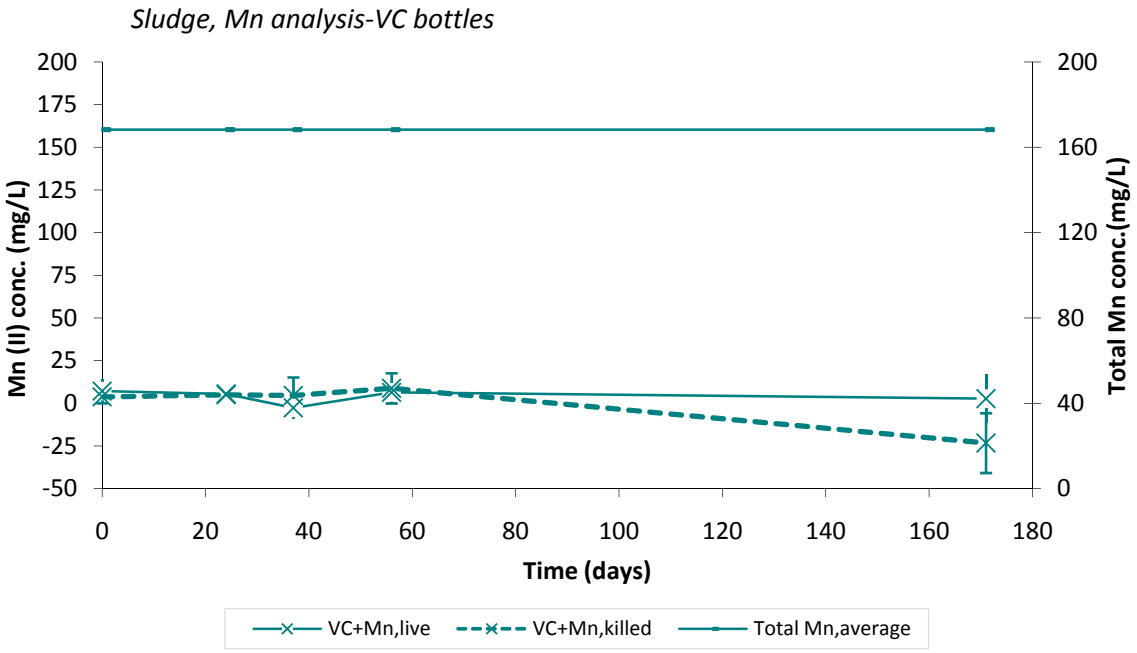

Sludge, VC+Mn
CONC. (umole/bottle)
Time (days)
VC+Mn live
VC+Mn killed


Sludge, VC w/o Fe/Mn
CONC. (umole/bottle)
Time (days)
VC live
VC killed

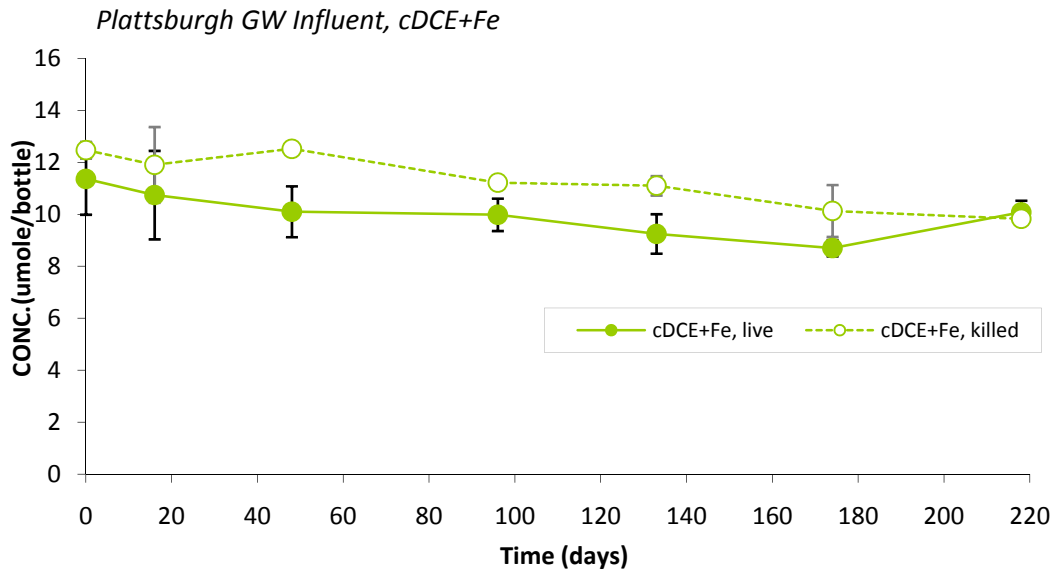

Sludge, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

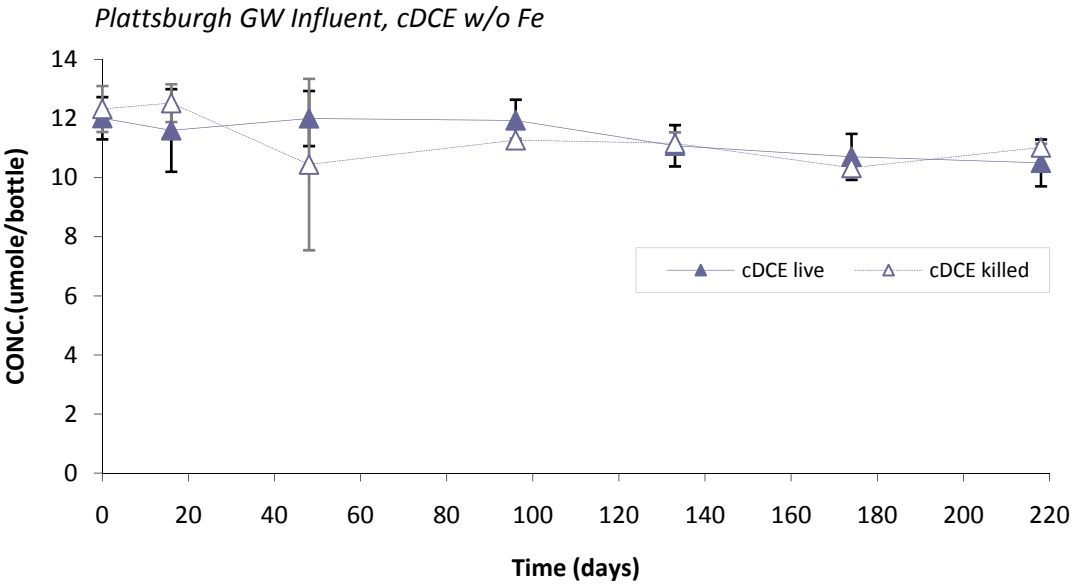

Sludge, Fe analysis-VC bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
VC+Fe, live
VC+Fe, killed
Total Fe,average

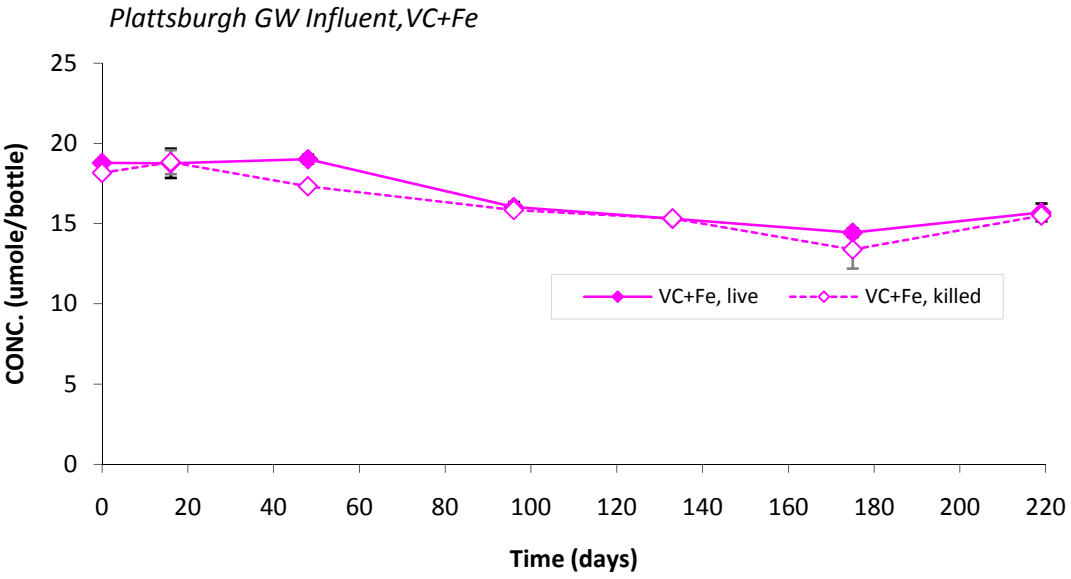

Sludge, Mn analysis-cDCE bottles
Mn (II) conc. (mg/L)
Total Mn conc.(mg/L)
Time (days)
cDCE+Mn,live
cDCE+Mn,killed
Total Mn,average

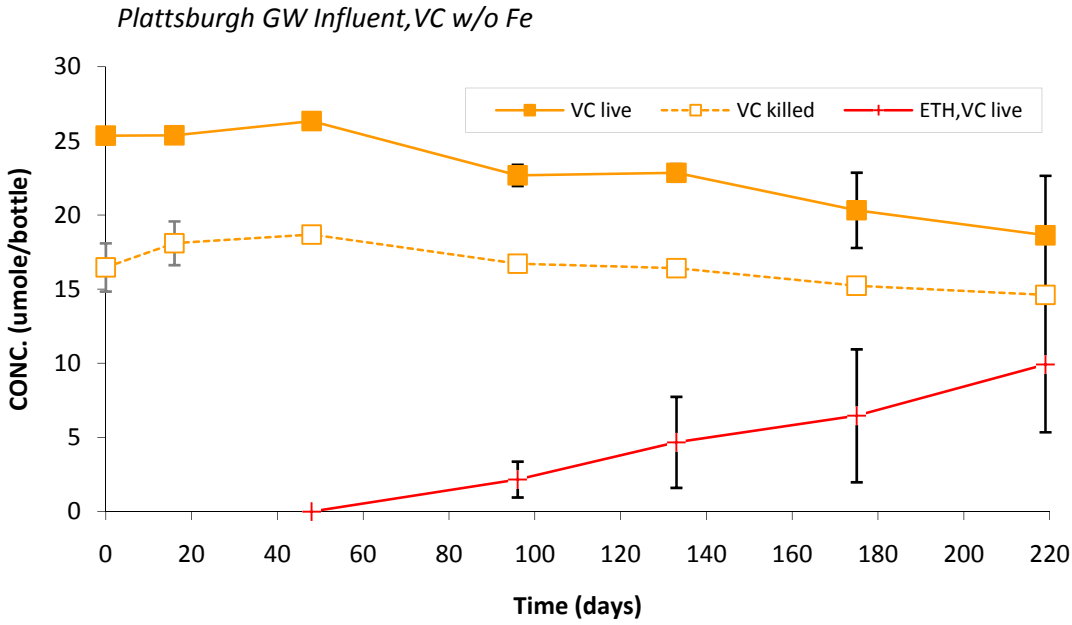


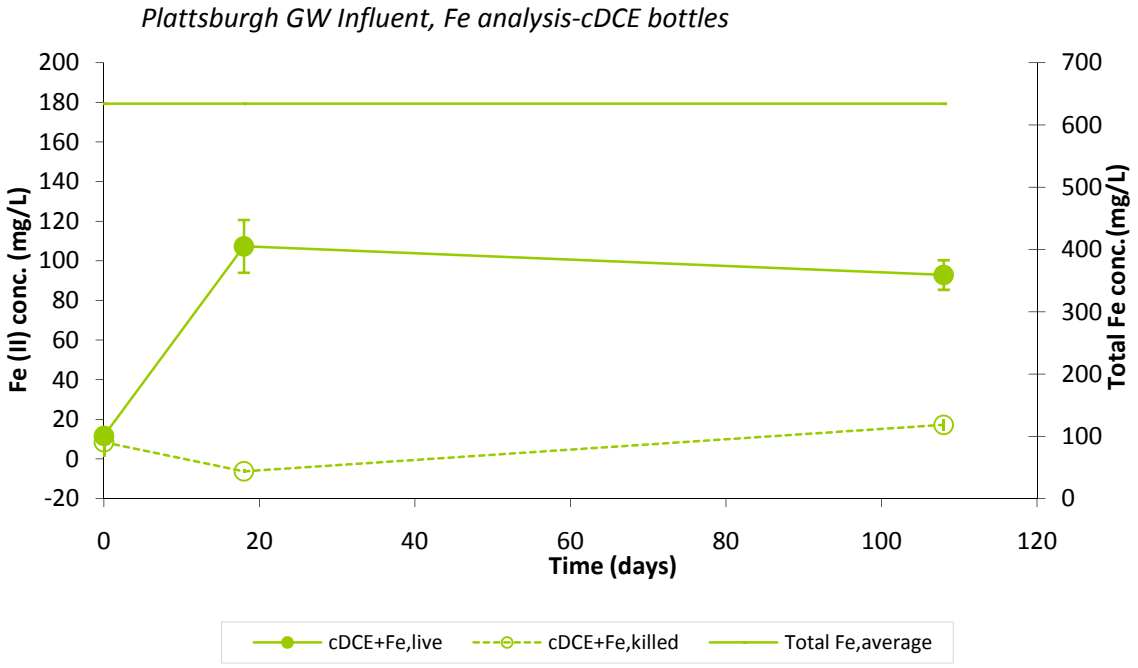
3-A. Site/Source 3: Plattsburgh AFB, Groundwater Influent:

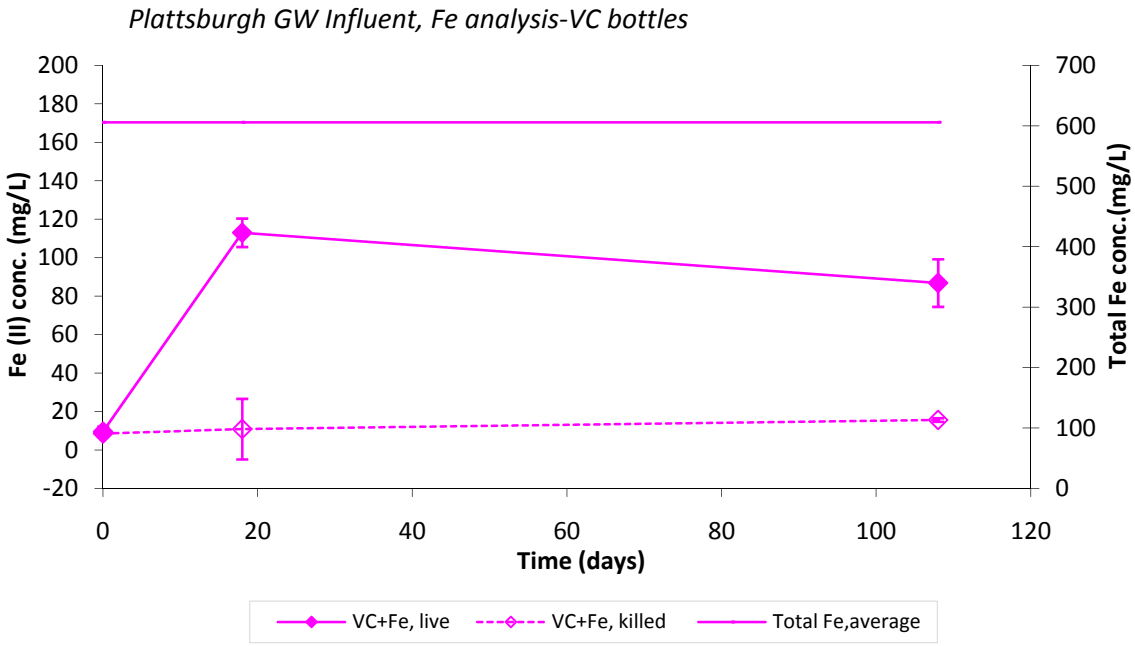



Plattsburgh GW Influent, cDCE w/o Fe
CONC.(umole/bottle)
Time (days)
cDCE live
cDCE killed
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120
140
160
180
200
220

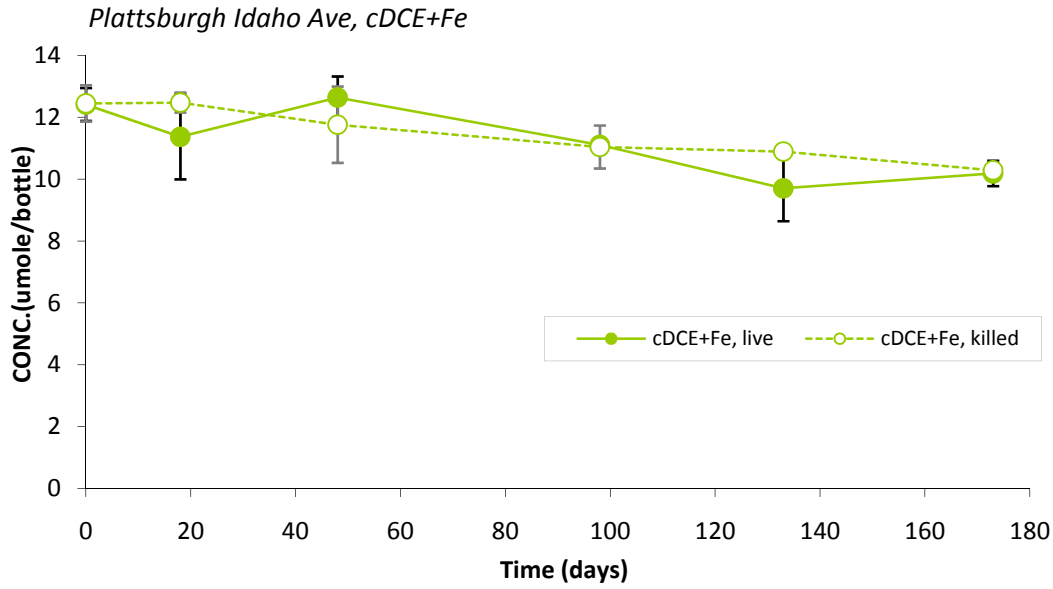

Plattsburgh GW Influent,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180
200
220

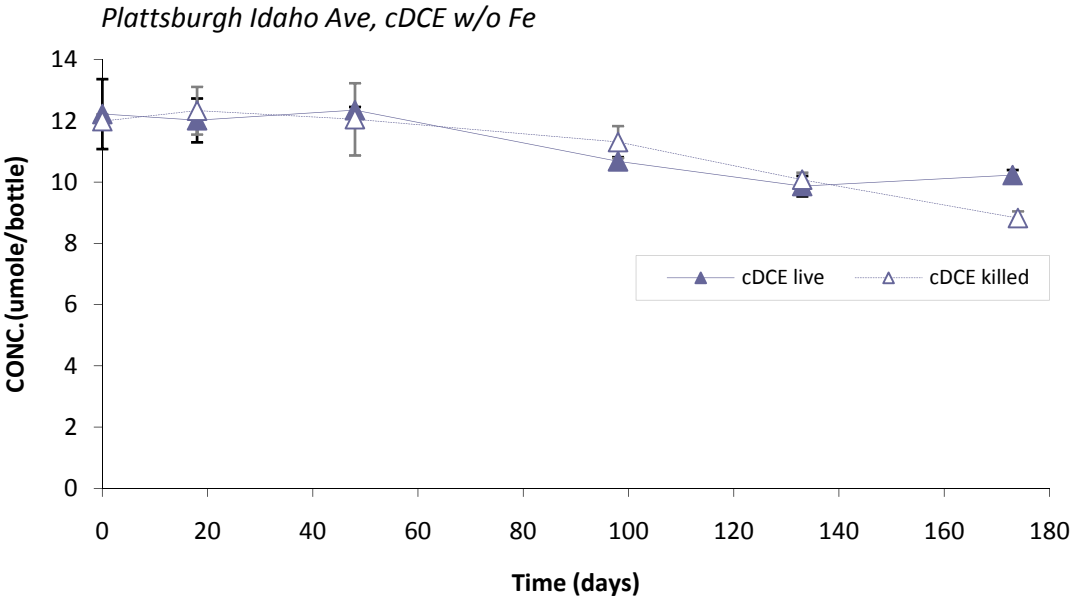

Plattsburgh GW Influent,VC w/o Fe
VC live
VC killed
ETH,VC live
CONC. (umole/bottle)
Time (days)
0
5
10
15
20
25
30
0
20
40
60
80
100
120
140
160
180
200
220

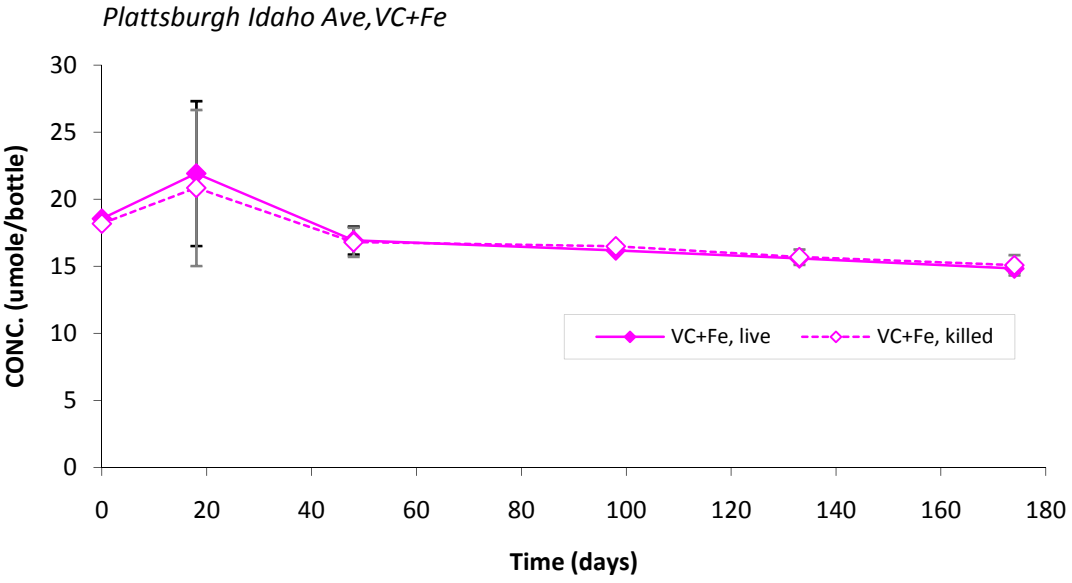

Plattsburgh GW Influent, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average
-20
0
20
40
60
80
100
120
140
160
180
200
0
100
200
300
400
500
600
700
0
20
40
60
80
100
120

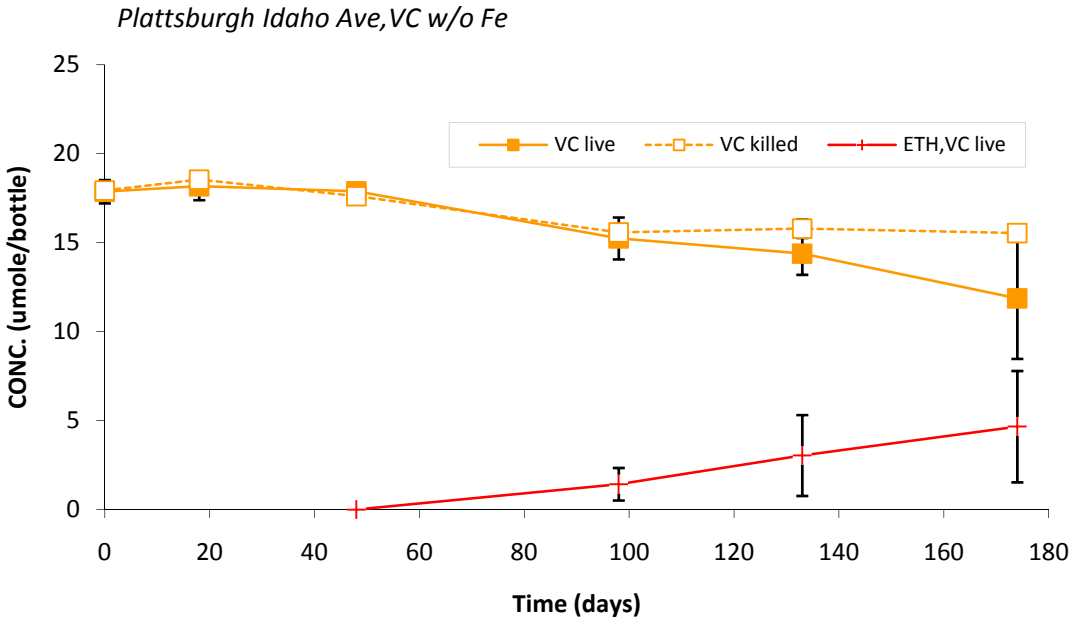


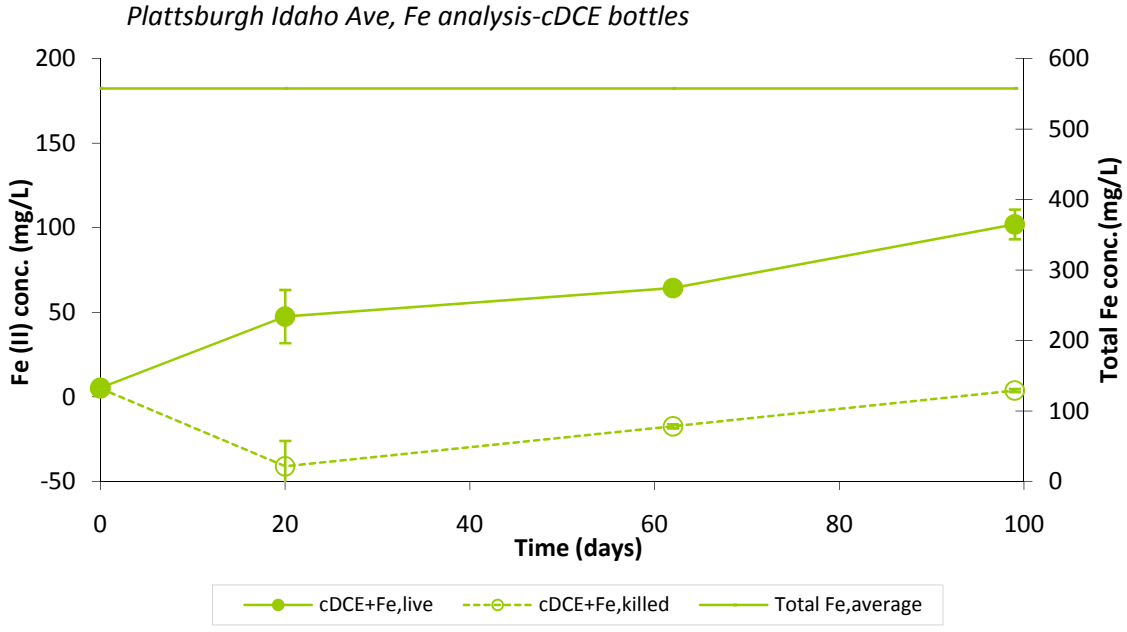
3-B. Site/Source 3: Plattsburgh AFB, Idaho Ave Discharge Pipe:

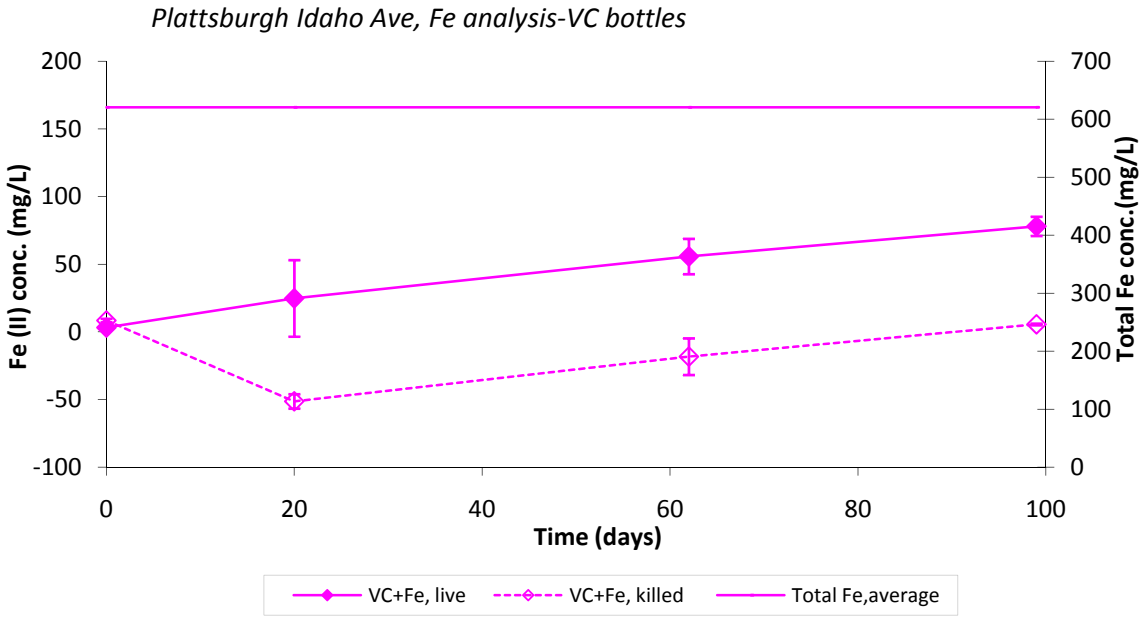



Plattsburgh Idaho Ave, cDCE w/o Fe
CONC.(umole/bottle)
Time (days)
cDCE live
cDCE killed
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120
140
160
180

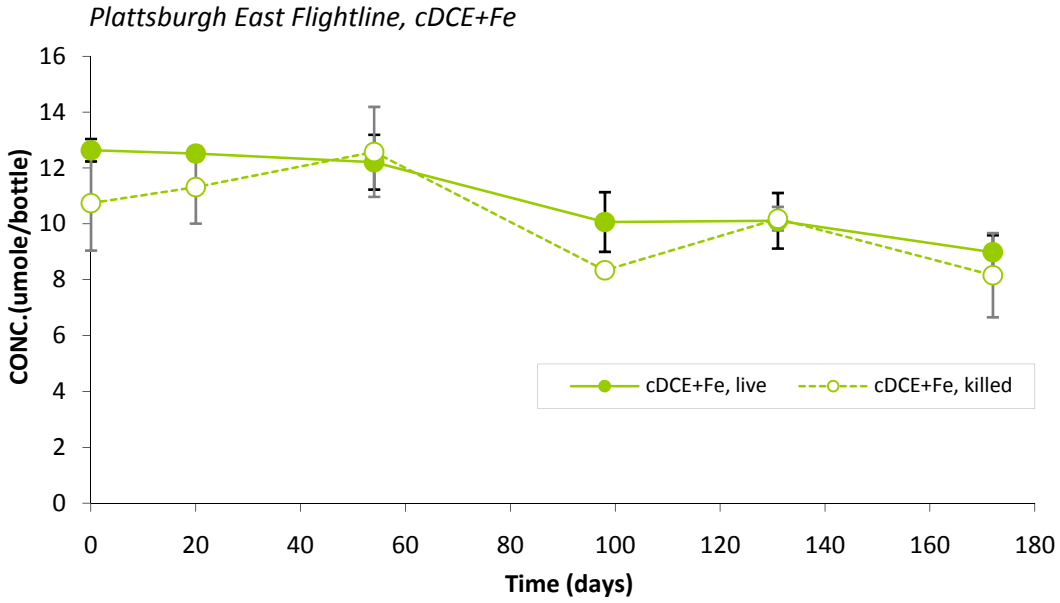

Plattsburgh Idaho Ave,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
5
10
15
20
25
30
0
20
40
60
80
100
120
140
160
180

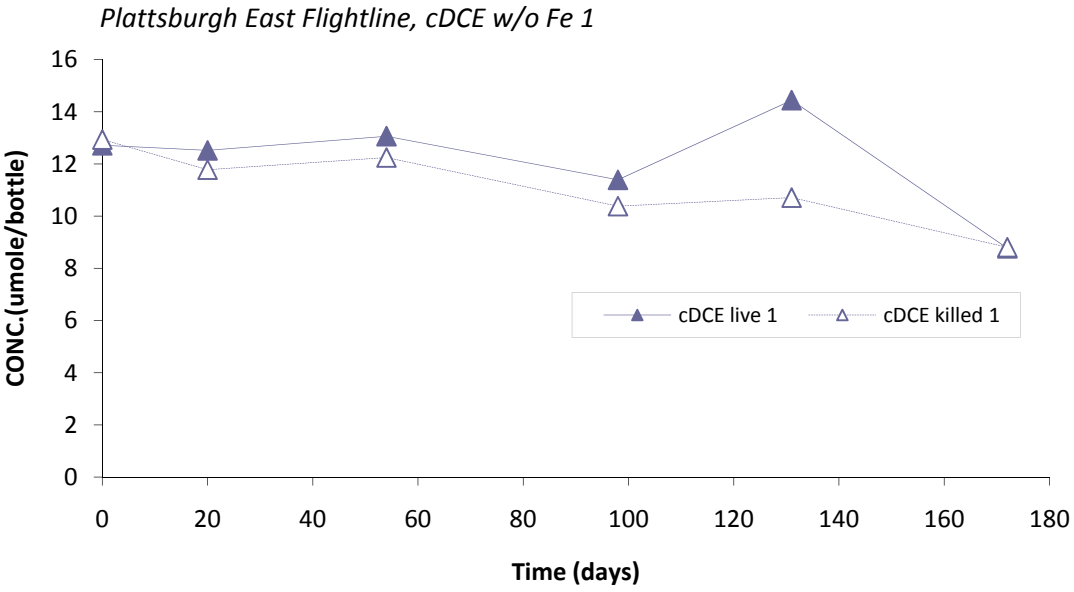

Plattsburgh Idaho Ave,VC w/o Fe
CONC. (umole/bottle)
Time (days)
VC live
VC killed
ETH,VC live
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180

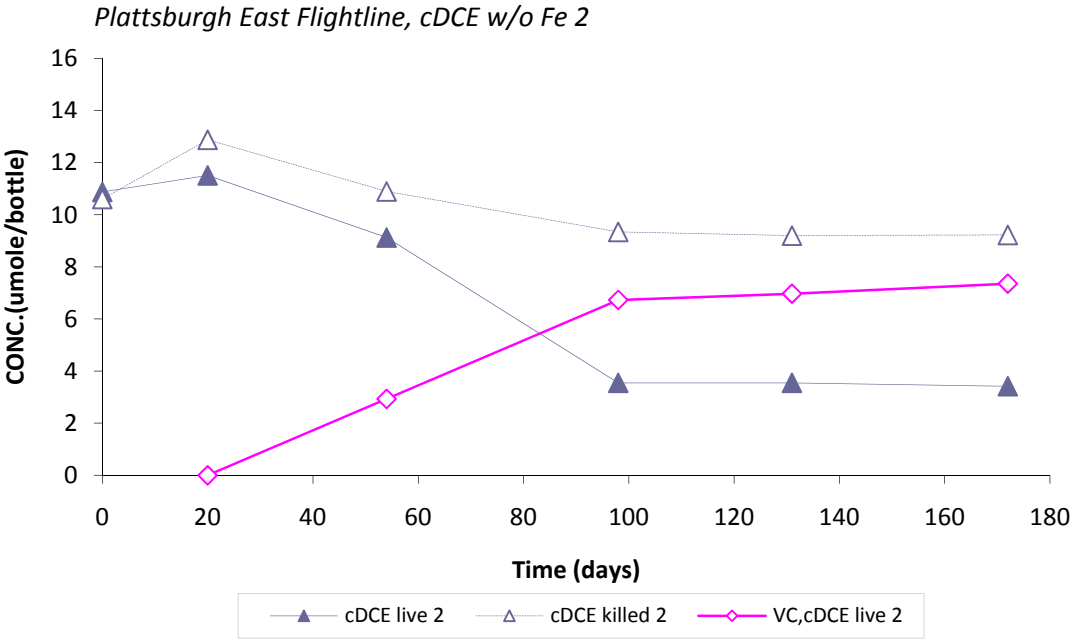

Plattsburgh Idaho Ave, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average
-50
0
50
100
150
200
0
100
200
300
400
500
600
0
20
40
60
80
100

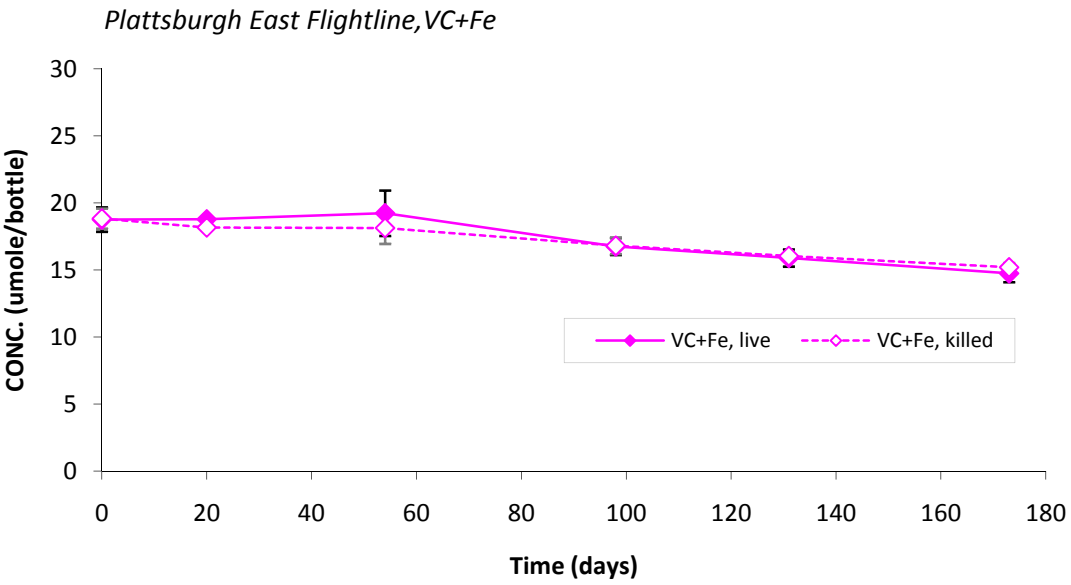


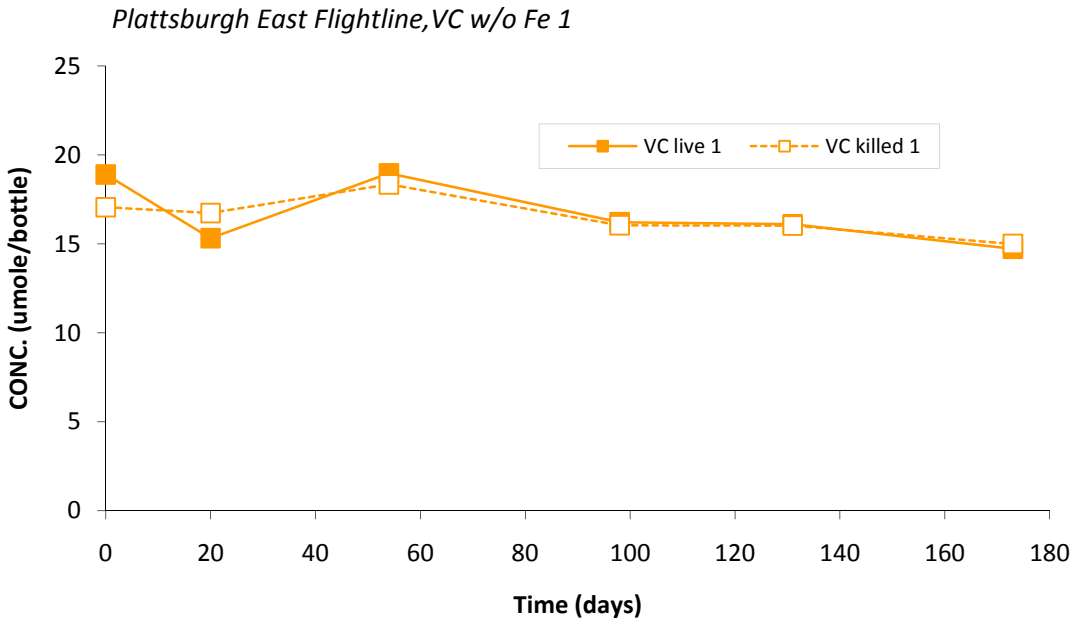
3-C. Site/Source 3: Plattsburgh AFB, East Flightline Discharge Pipe:

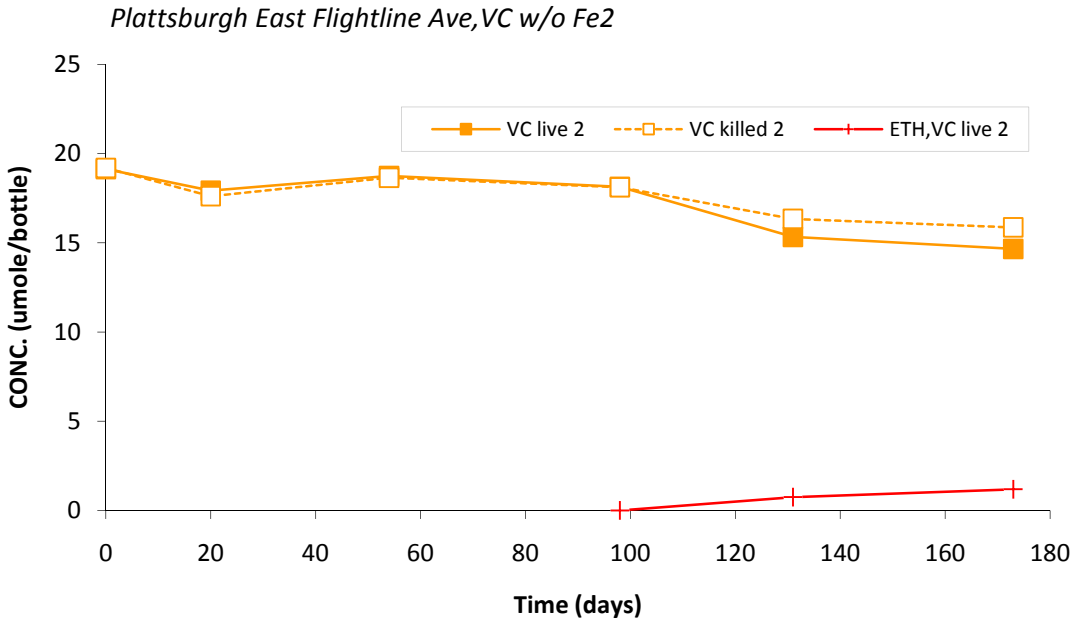


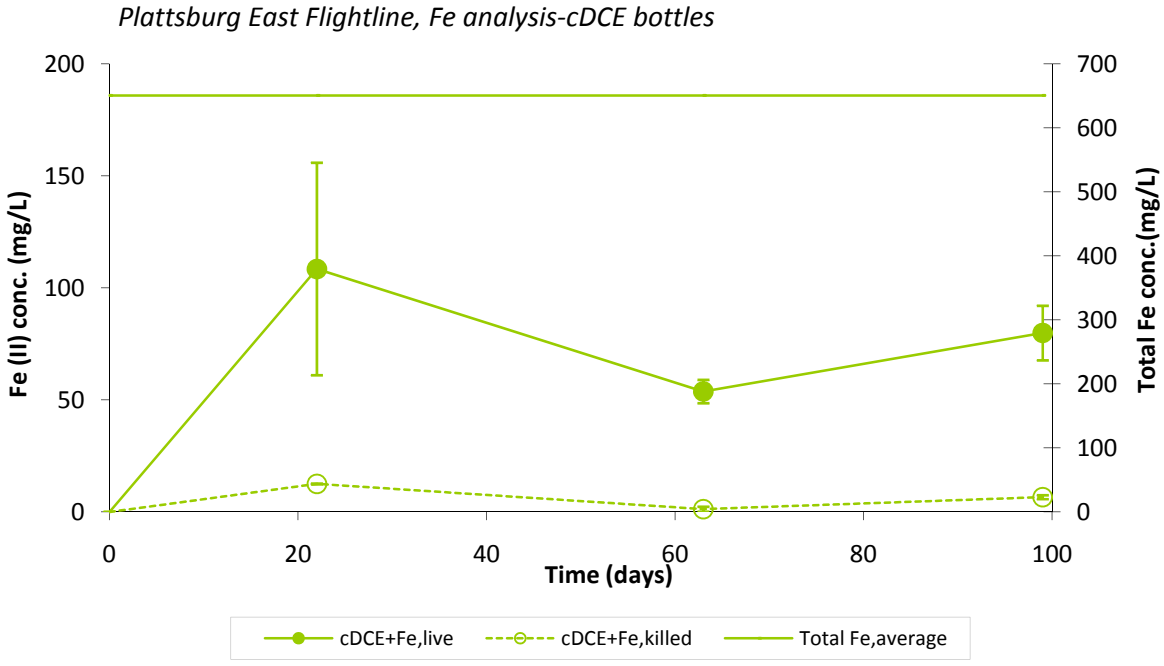

Plattsburgh East Flightline, cDCE w/o Fe 1
CONC.(umole/bottle)
Time (days)
cDCE live 1
cDCE killed 1

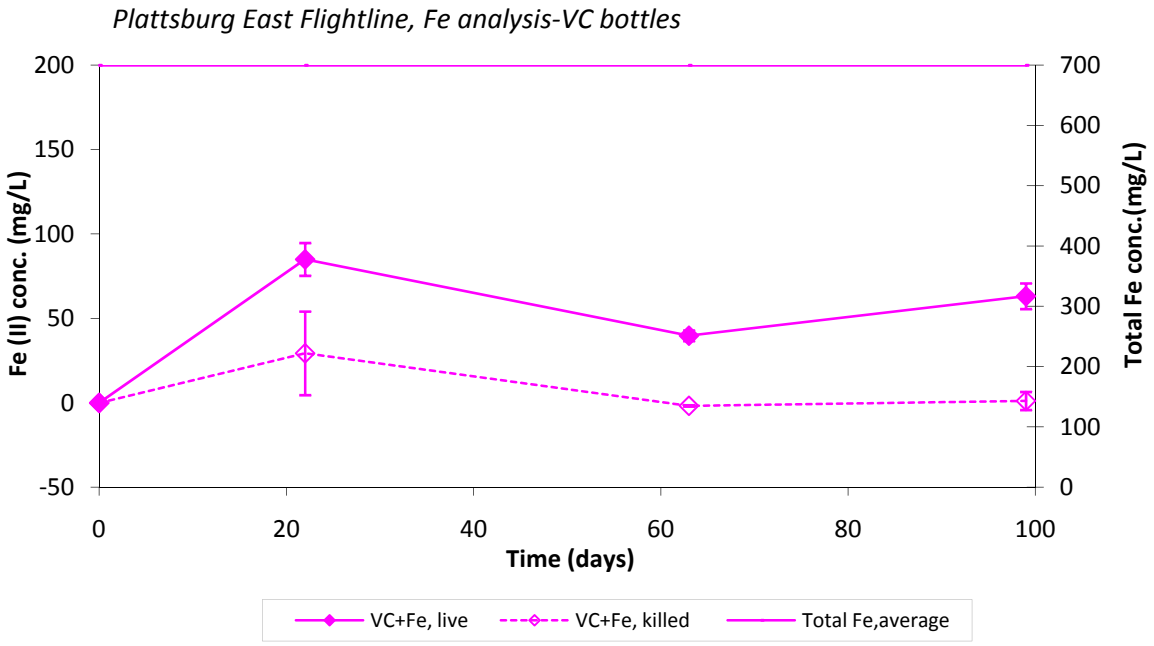

Plattsburgh East Flightline, cDCE w/o Fe 2
CONC.(umole/bottle)
Time (days)
cDCE live 2
cDCE killed 2
VC,cDCE live 2


Plattsburgh East Flightline,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
5
10
15
20
25
30
0
20
40
60
80
100
120
140
160
180

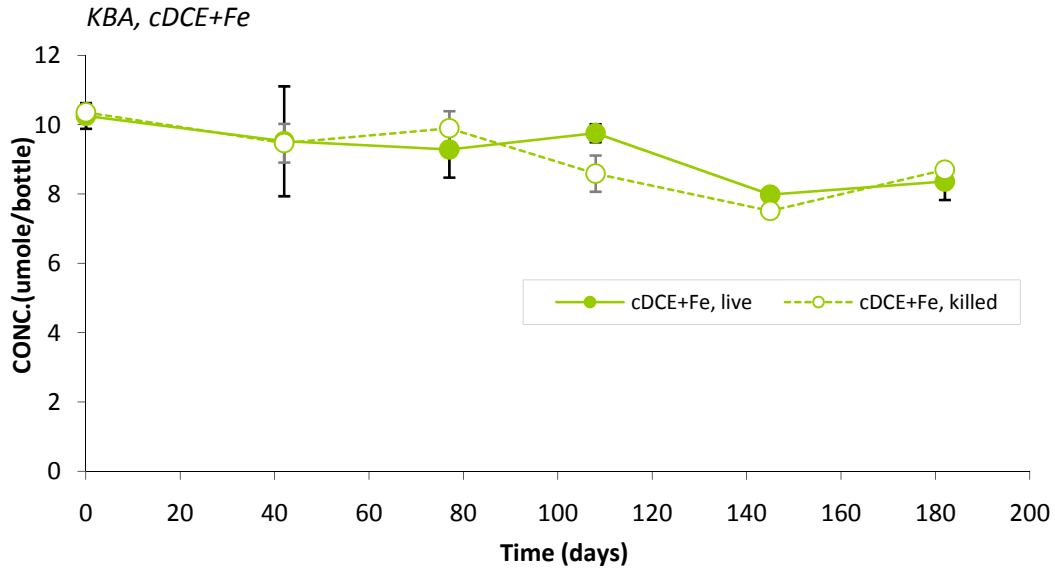

Plattsburgh East Flightline,VC w/o Fe 1
CONC. (umole/bottle)
Time (days)
VC live 1
VC killed 1
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180

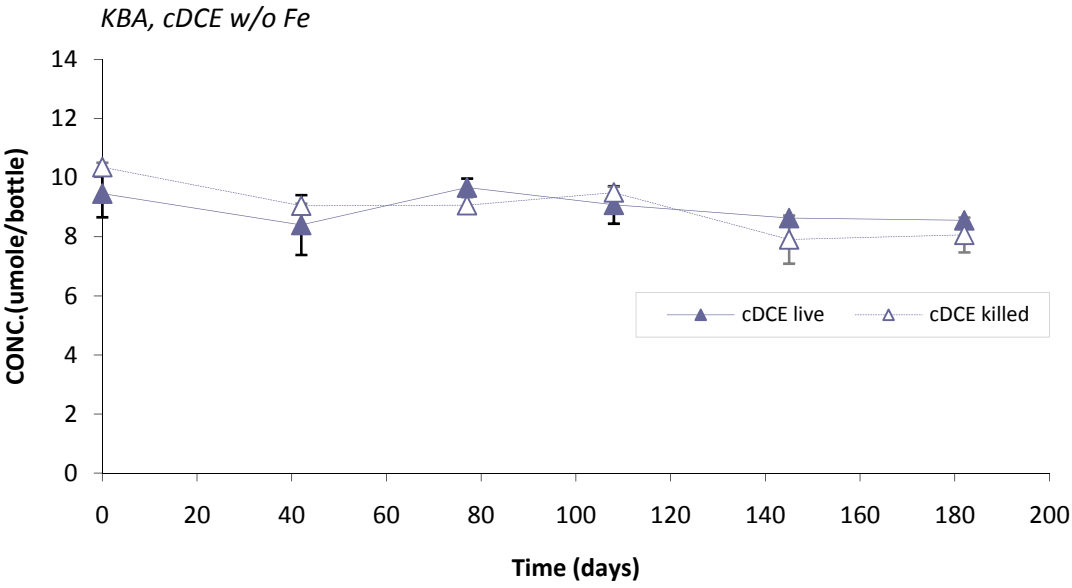

Plattsburgh East Flightline Ave,VC w/o Fe2
CONC. (umole/bottle)
Time (days)
VC live 2
VC killed 2
ETH,VC live 2
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180

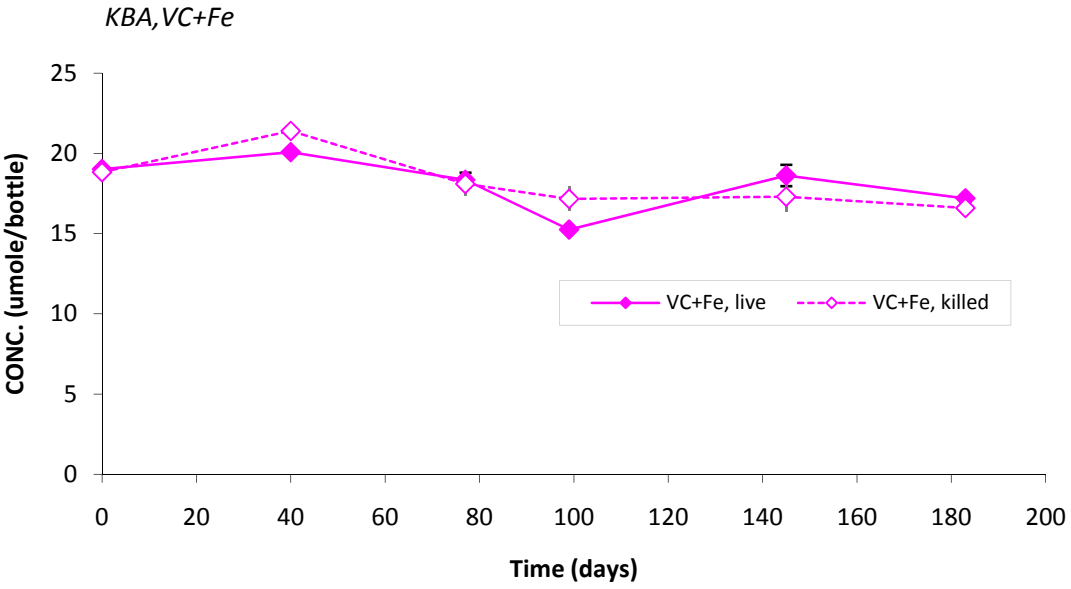

Plattsburg East Flightline, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average
0
50
100
150
200
0
100
200
300
400
500
600
700
0
20
40
60
80
100

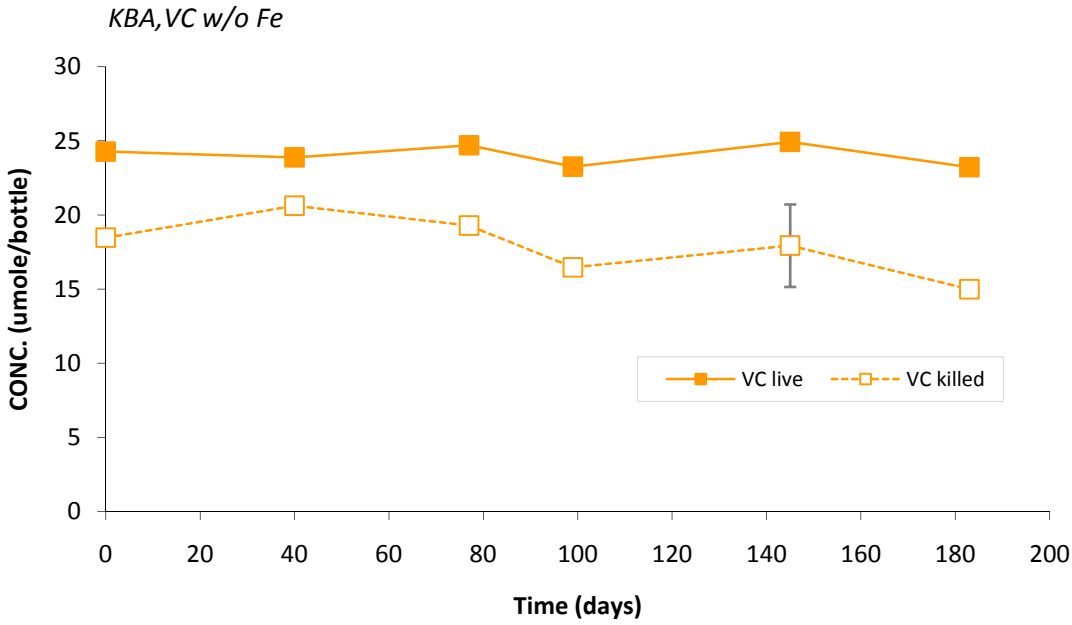


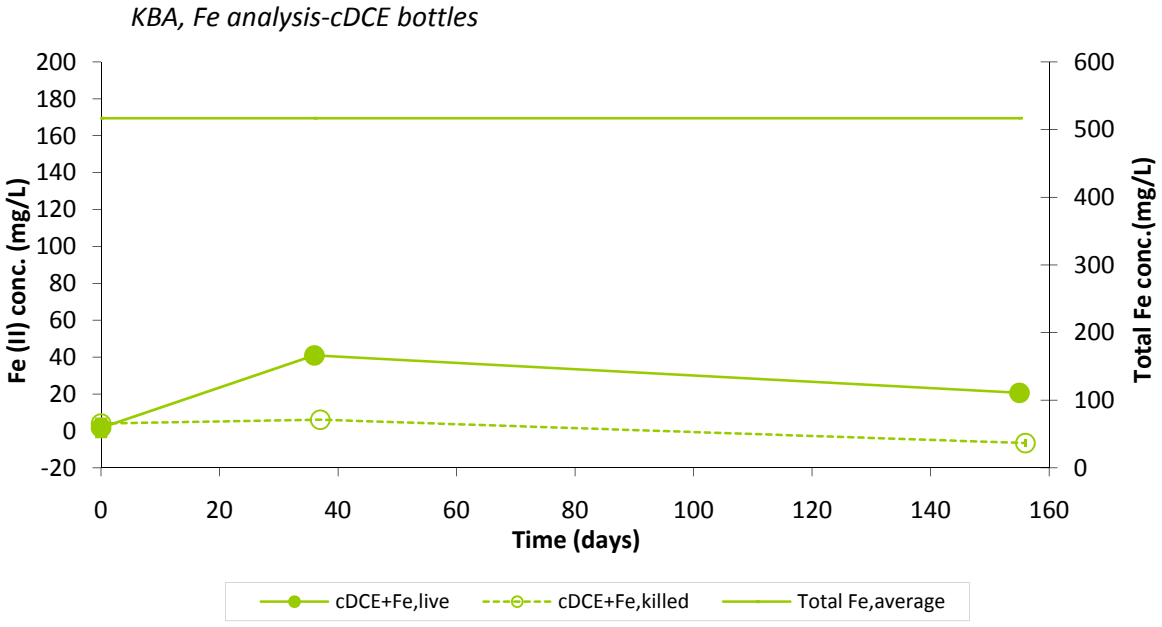
4-A. Site/Source 4: NSB, Kings Bay KBA-11-13A:

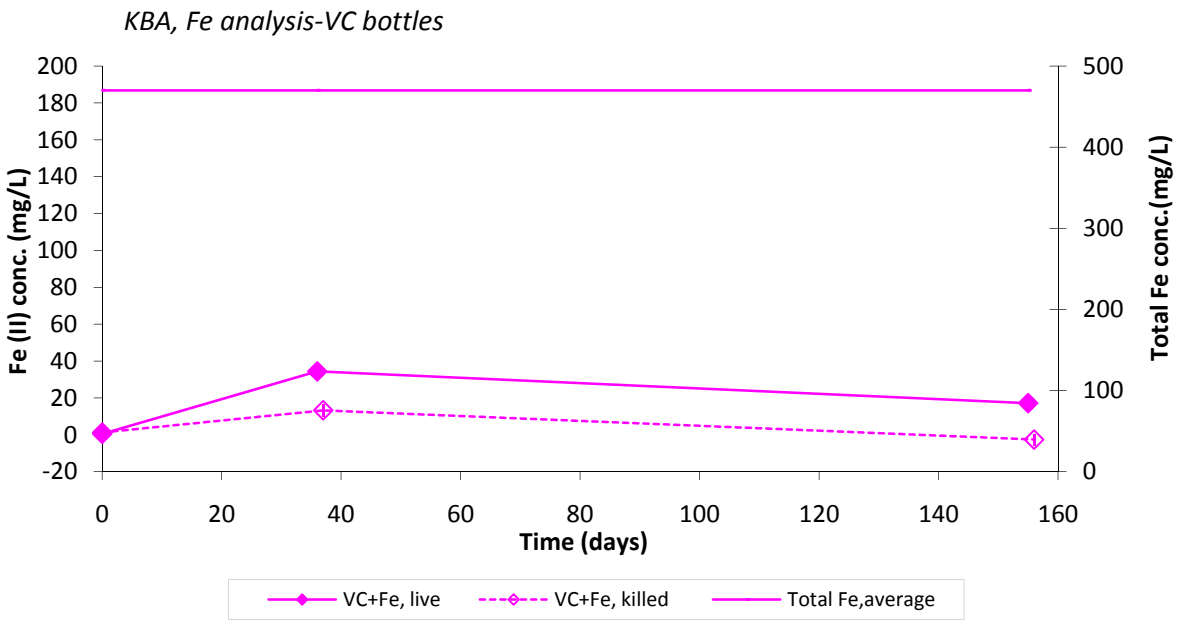



KBA, cDCE w/o Fe
CONC.(umole/bottle)
Time (days)
cDCE live
cDCE killed
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120
140
160
180
200

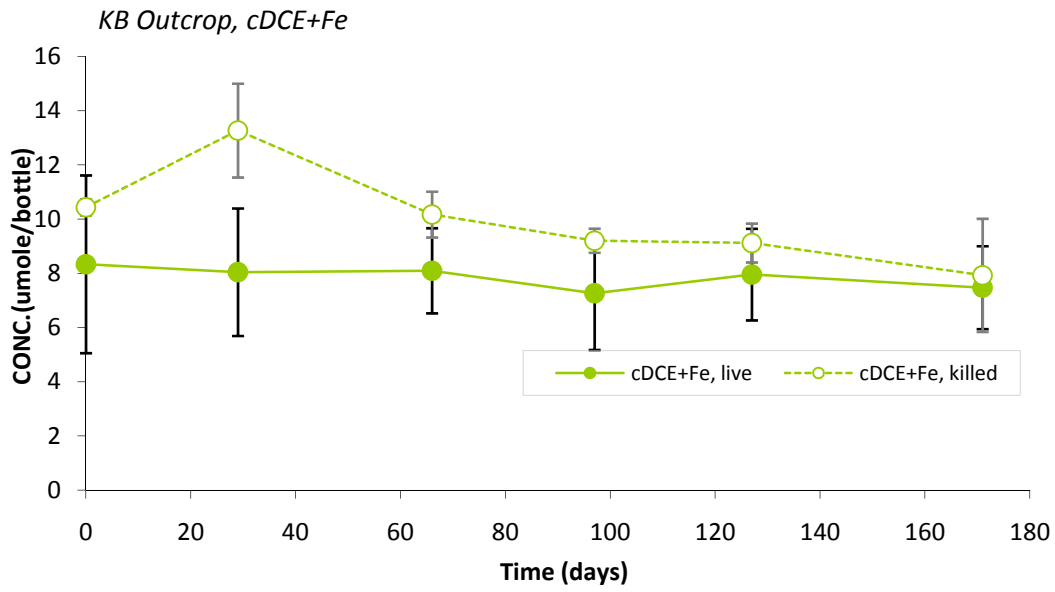

KBA,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180
200

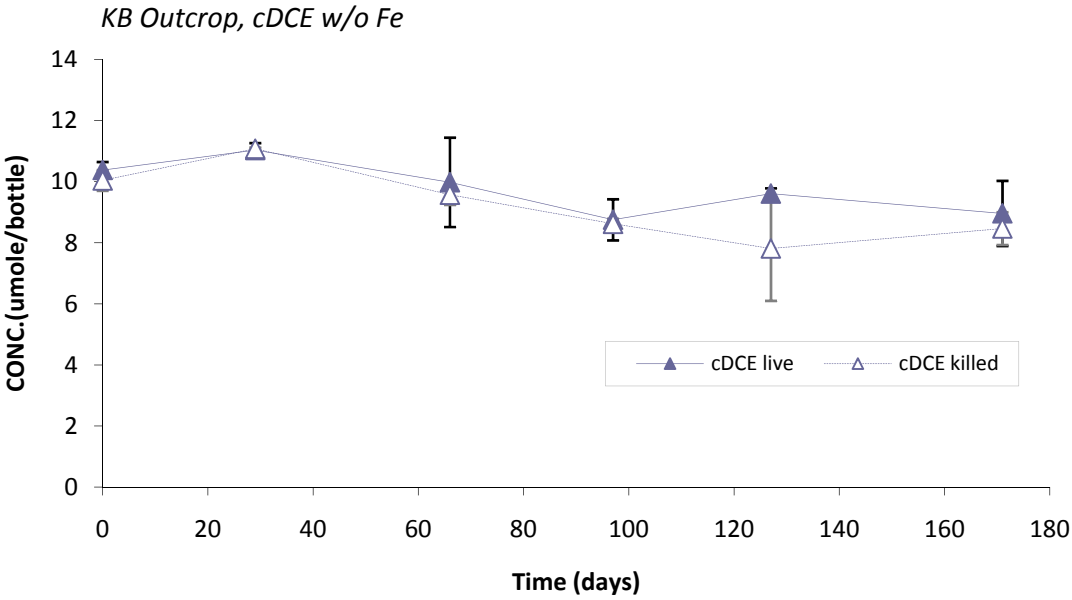

KBA,VC w/o Fe
CONC. (umole/bottle)
Time (days)
VC live
VC killed

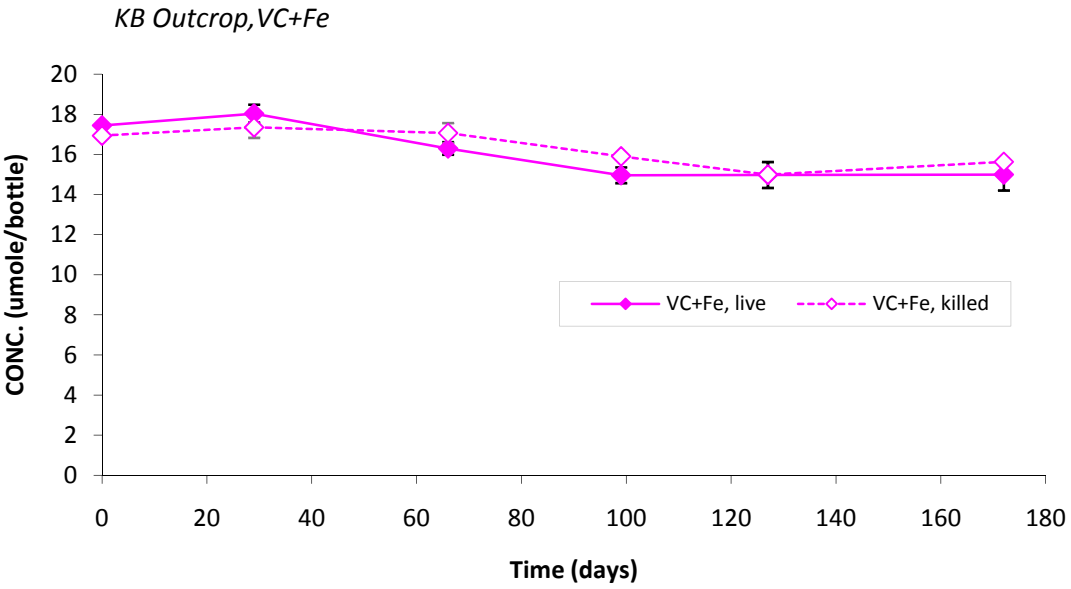

KBA, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

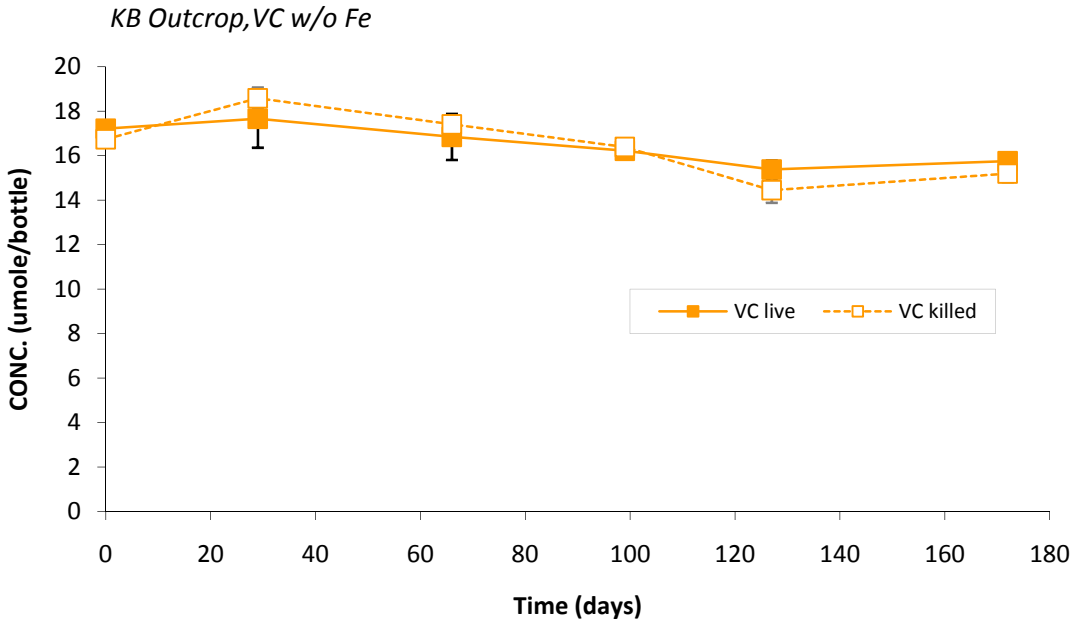


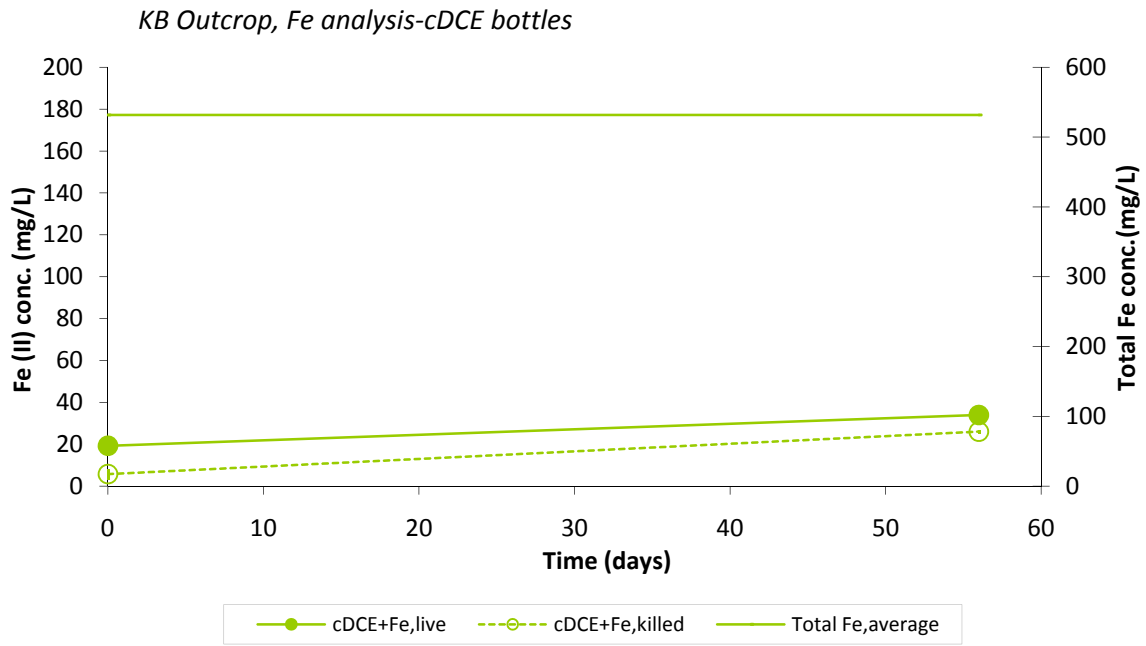
4-B. Site/Source 4: NSB, Kings Bay Outcrop:

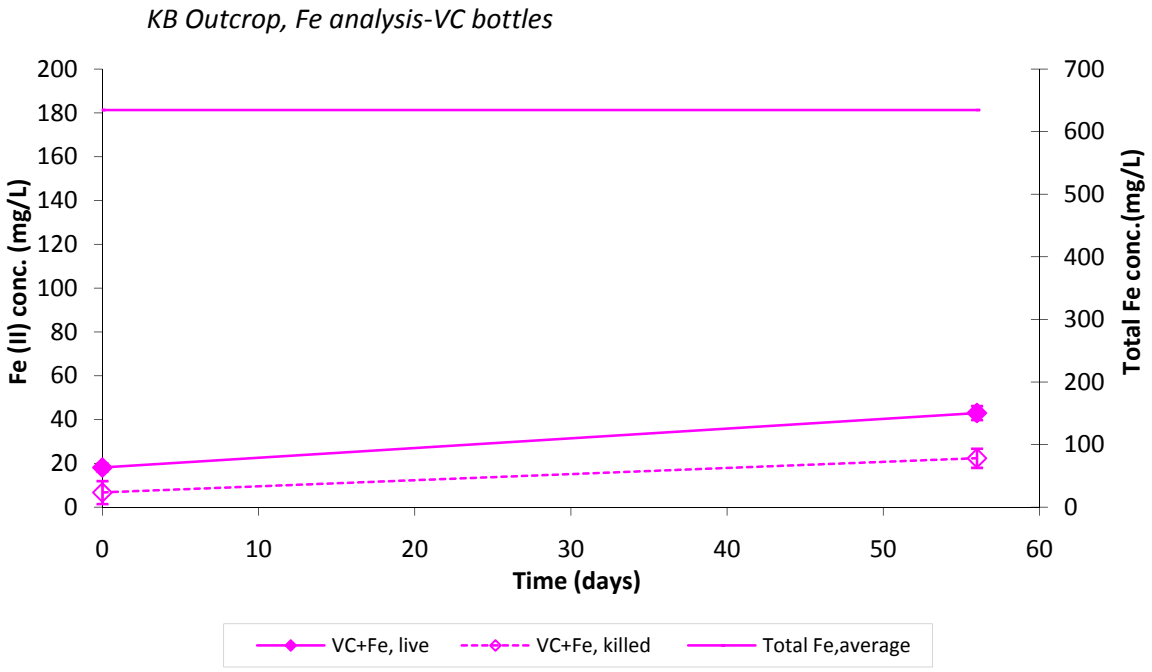



KB Outcrop, cDCE w/o Fe
CONC.(umole/bottle)
Time (days)
cDCE live
cDCE killed
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120
140
160
180

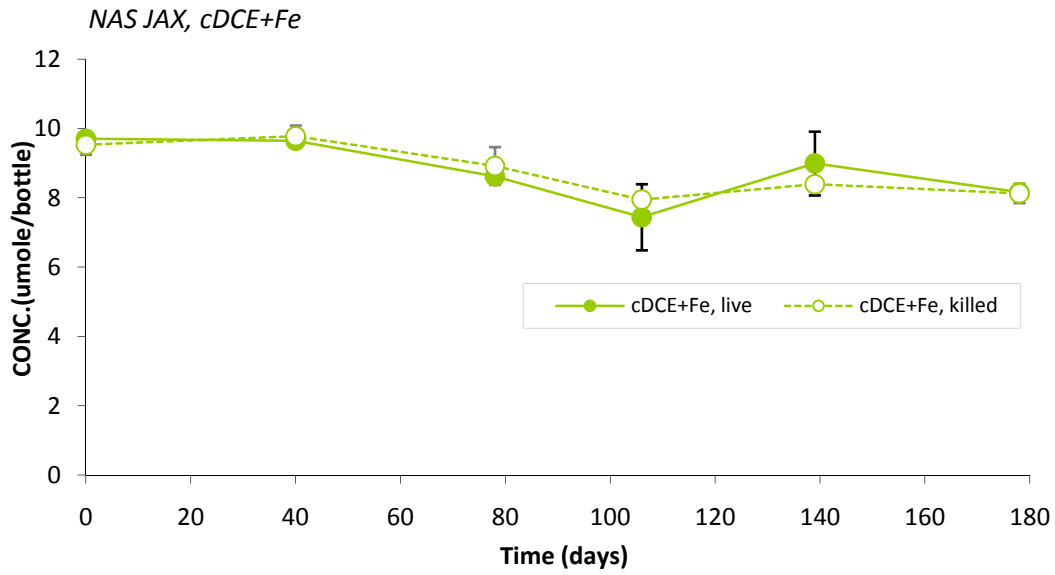

KB Outcrop,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100
120
140
160
180

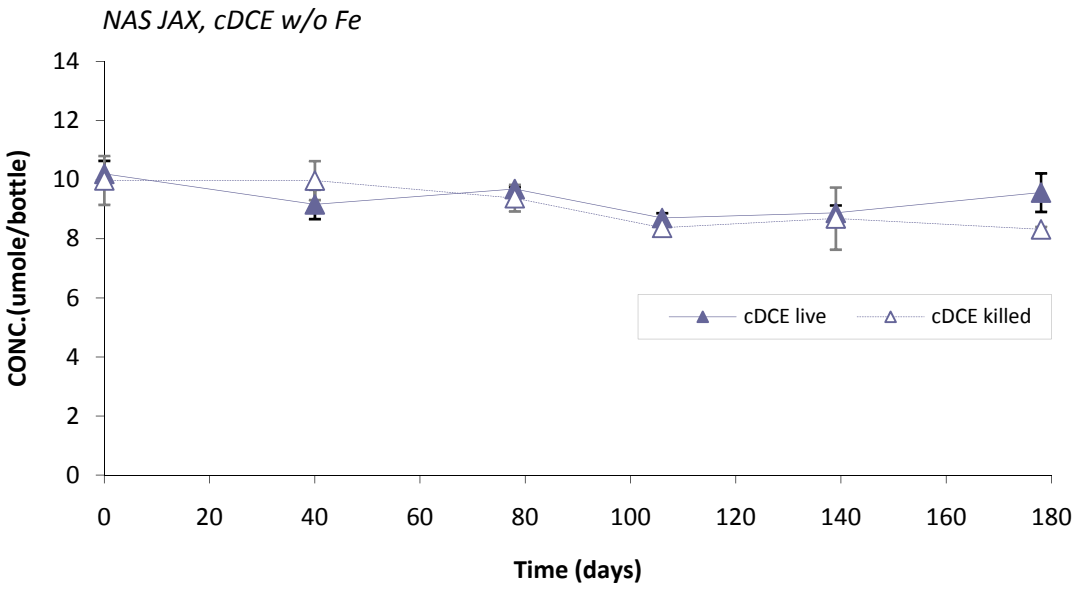

KB Outcrop,VC w/o Fe
CONC. (umole/bottle)
Time (days)
VC live
VC killed

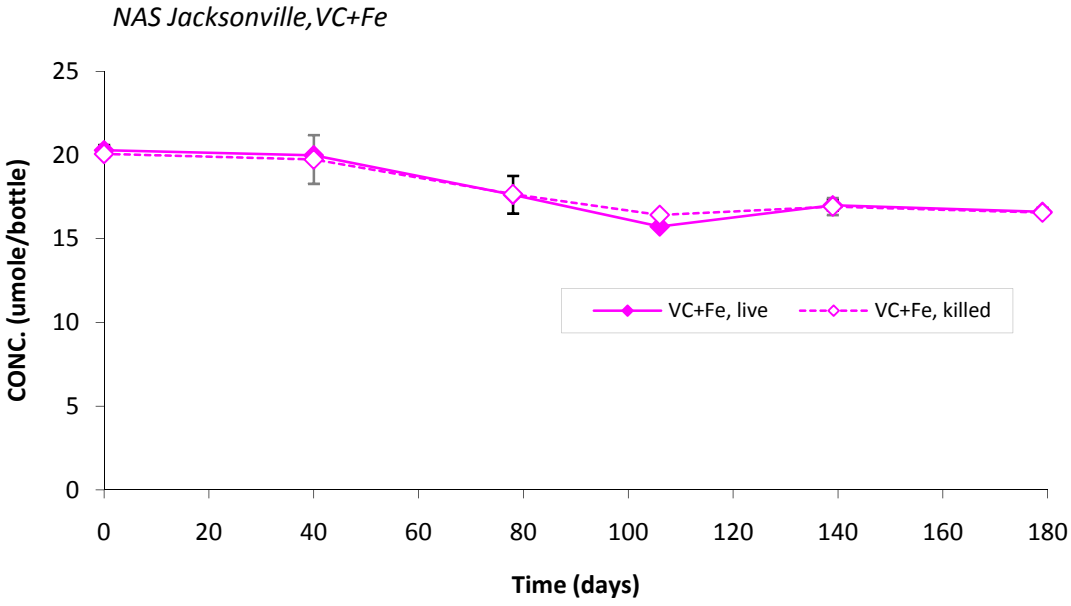

KB Outcrop, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

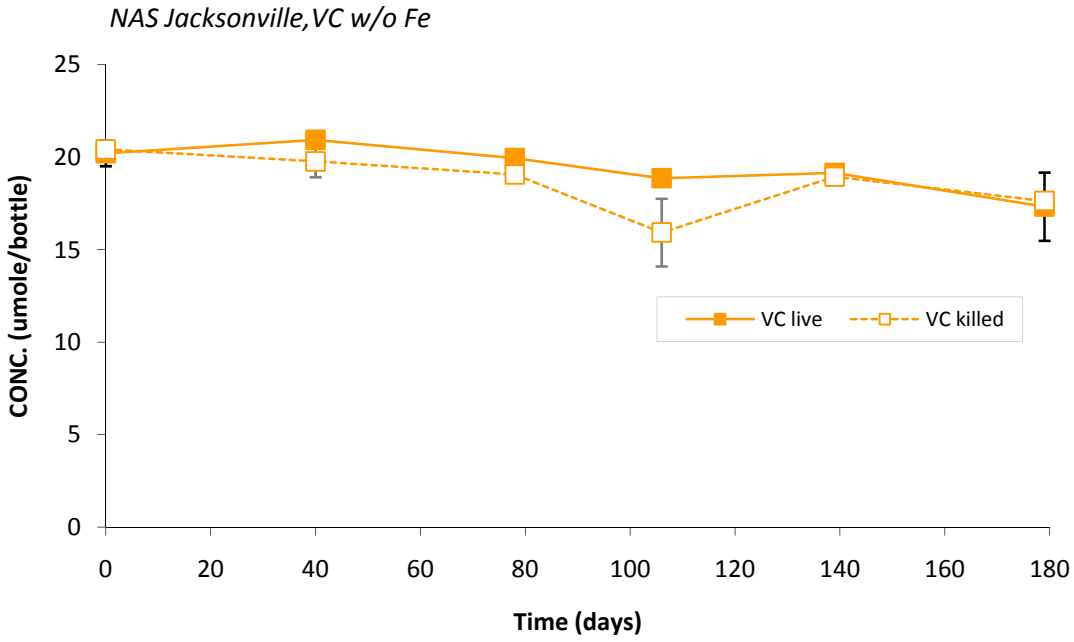


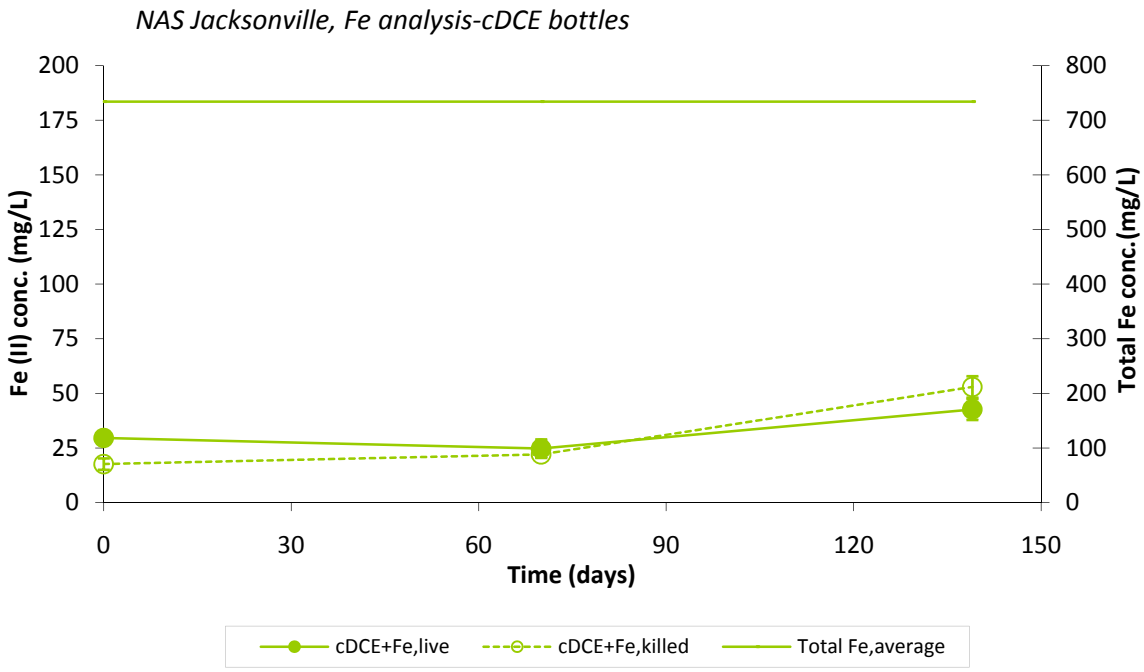
5-A. Site/Source 5: Naval Air Station, Jacksonville:

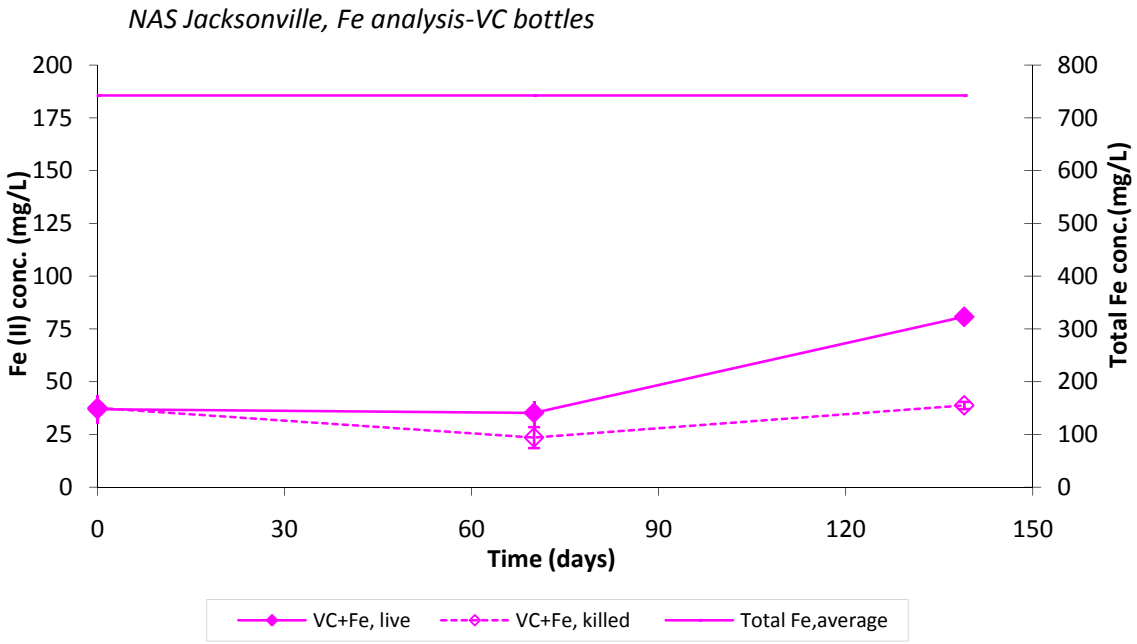



NAS JAX, cDCE w/o Fe
CONC.(umole/bottle)
Time (days)
cDCE live
cDCE killed

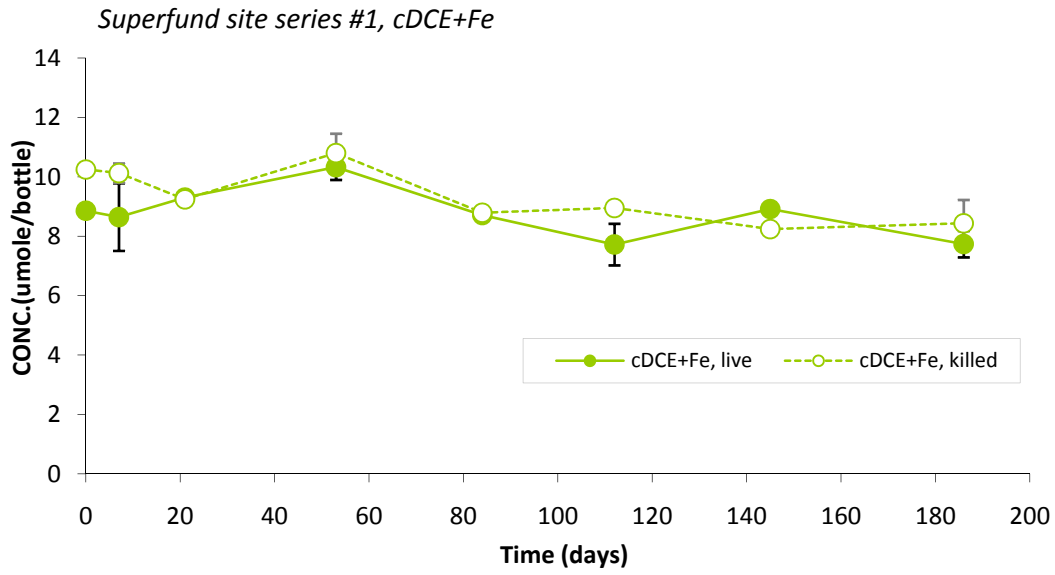

NAS Jacksonville,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed

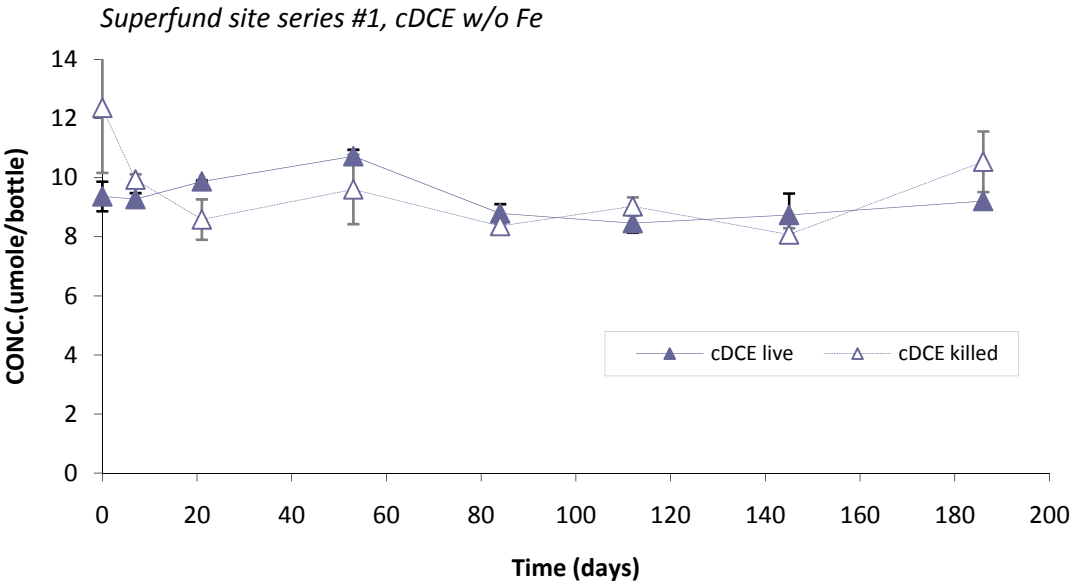

NAS Jacksonville,VC w/o Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
180
Time (days)
VC live
VC killed

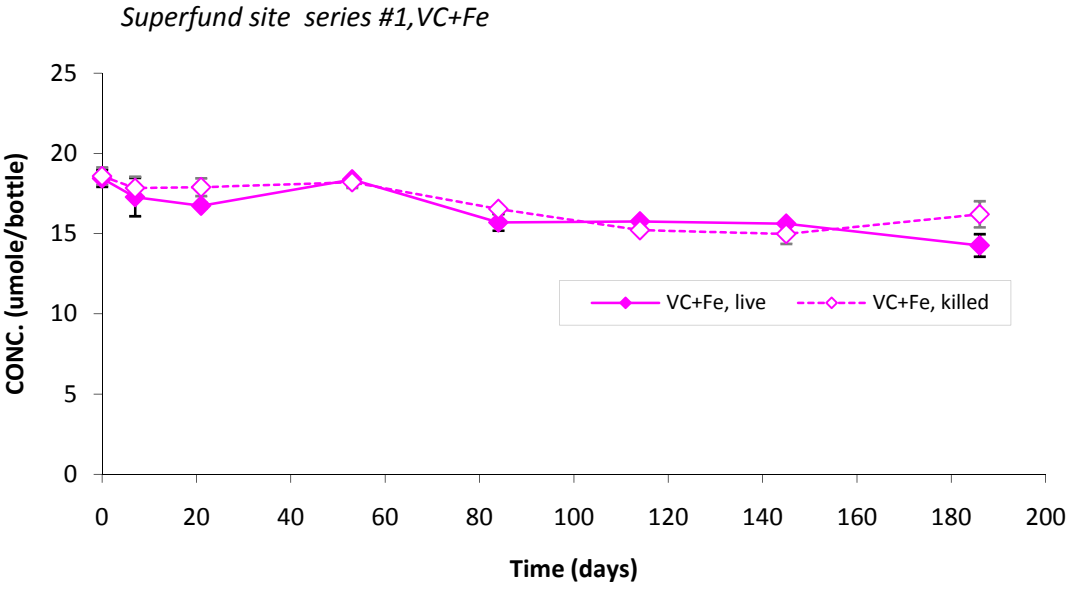

NAS Jacksonville, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
200
175
150
125
100
75
50
25
0
Total Fe conc.(mg/L)
800
700
600
500
400
300
200
100
0
0
30
60
90
120
150
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

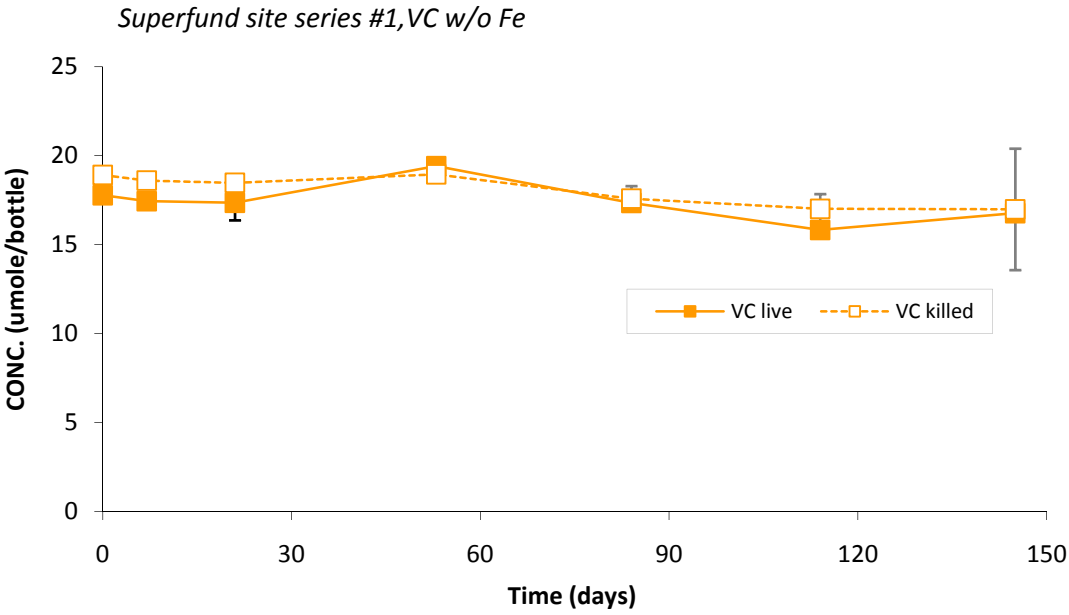


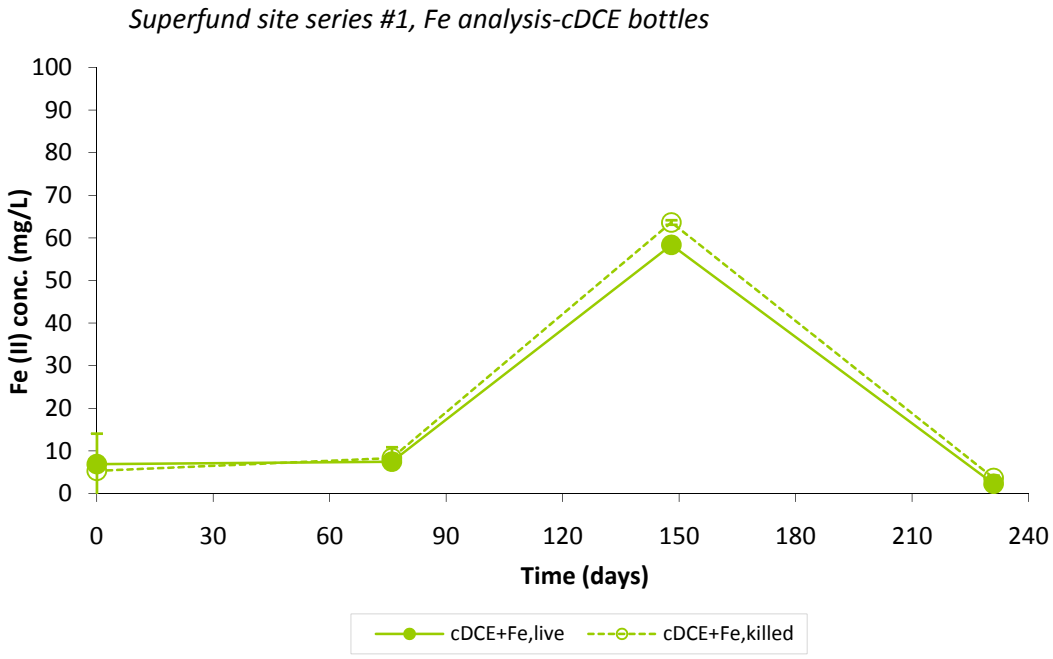
6-A. Site/Source 6: Undisclosed Superfund Site Series #1:

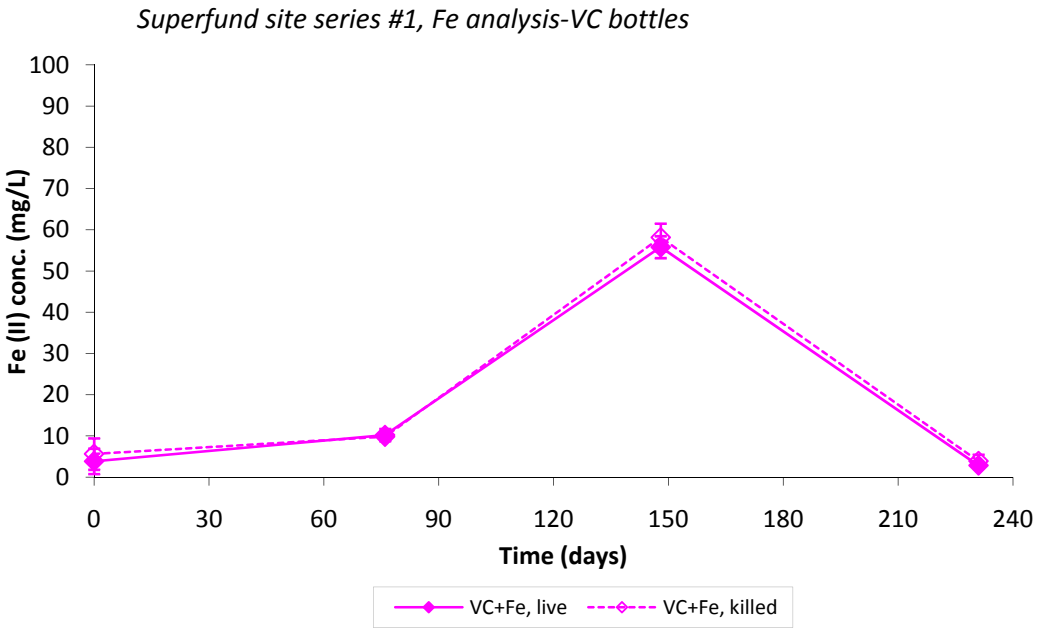



Superfund site series #1, cDCE w/o Fe
CONC.(umole/bottle)
Time (days)
cDCE live
cDCE killed
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120
140
160
180
200

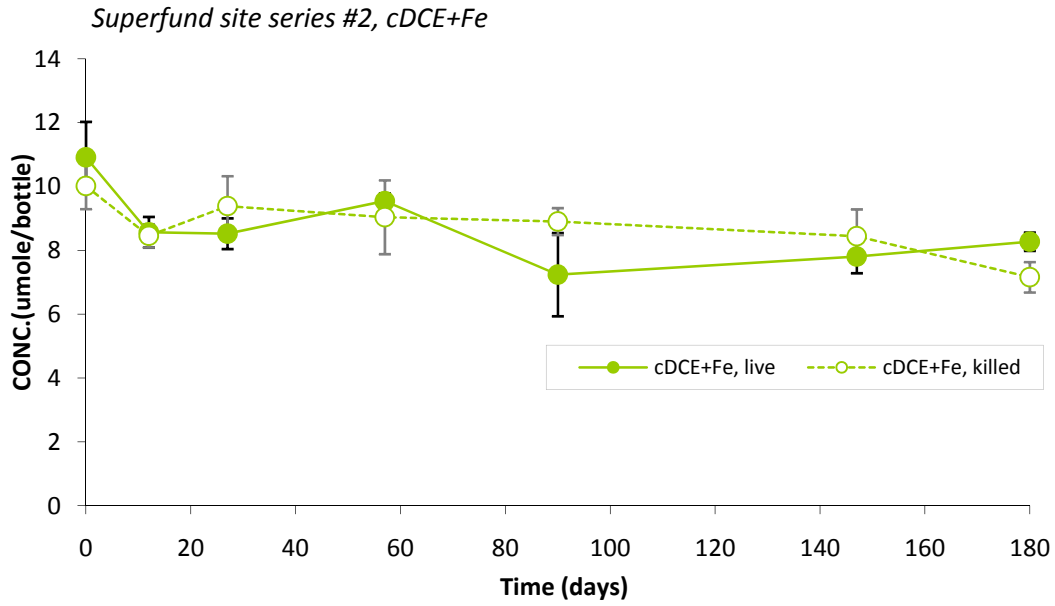

Superfund site series #1,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180
200

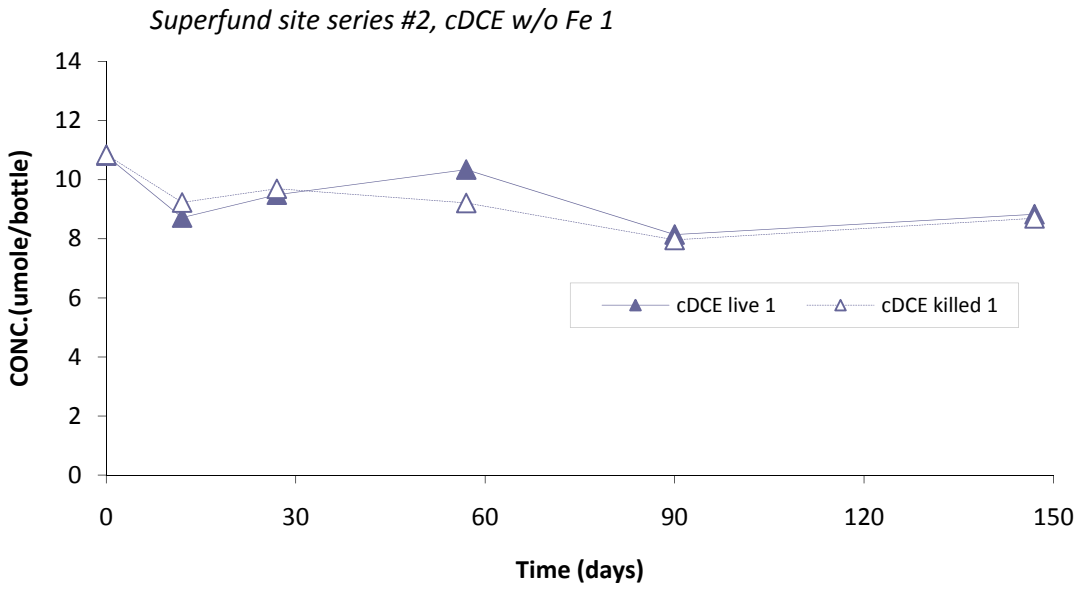

Superfund site series #1,VC w/o Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
30
60
90
120
150
Time (days)
VC live
VC killed

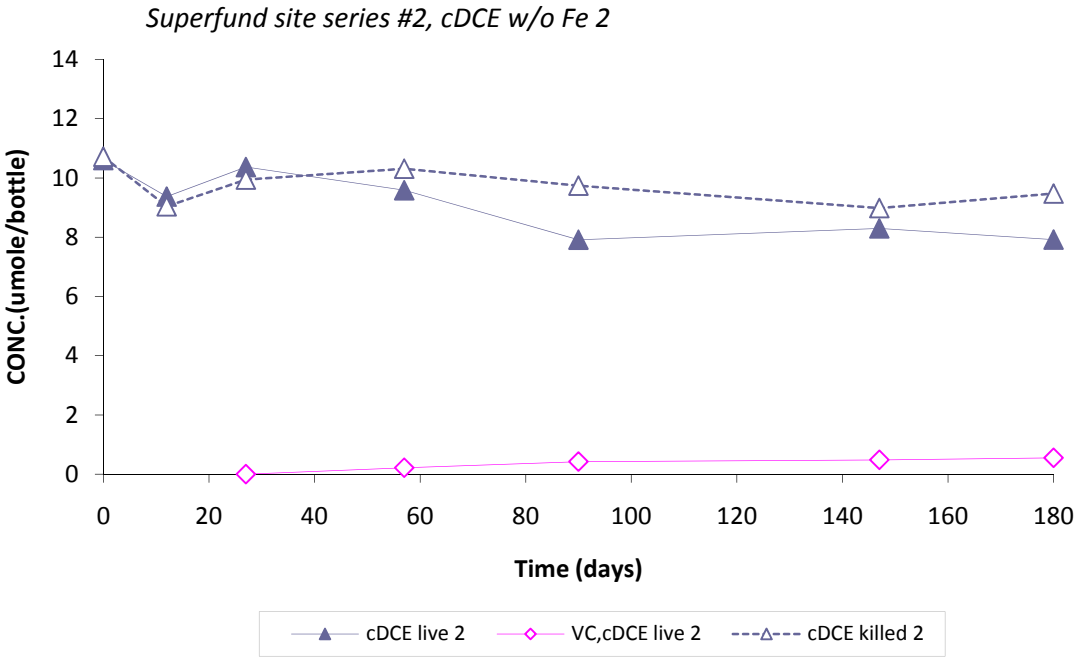

Superfund site series #1, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
100
90
80
70
60
50
40
30
20
10
0
0
30
60
90
120
150
180
210
240
Time (days)
cDCE+Fe,live
cDCE+Fe,killed

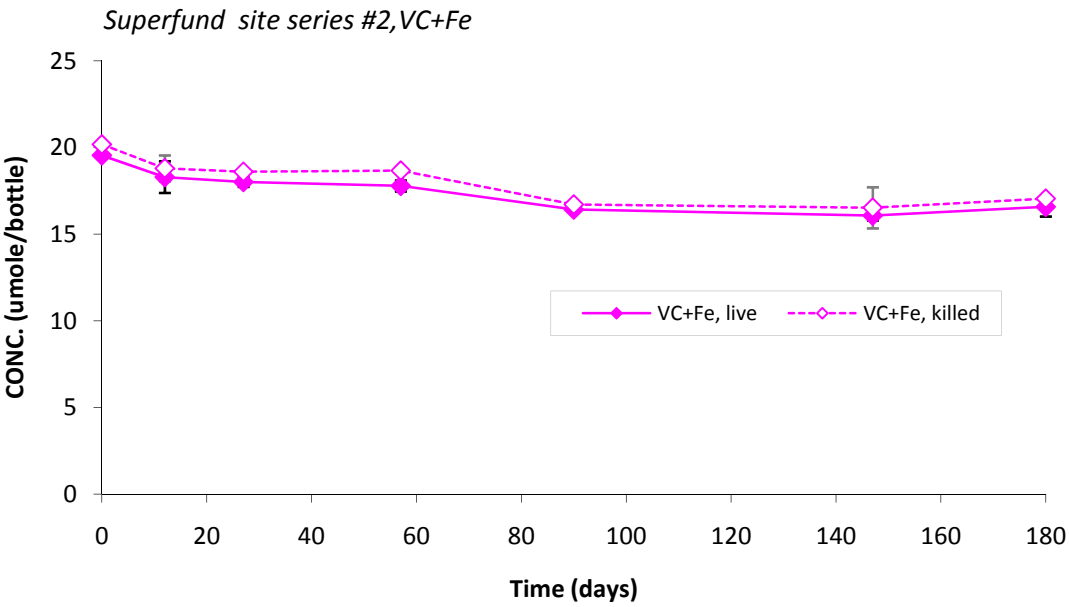


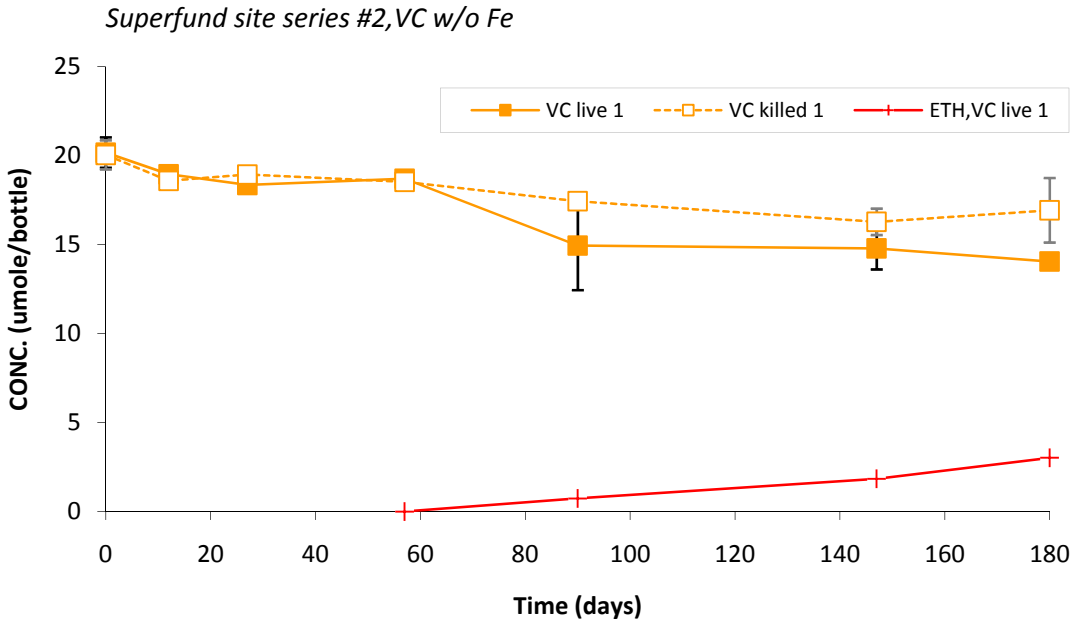
6-B. Site/Source 6: Undisclosed Superfund Site Series #2:

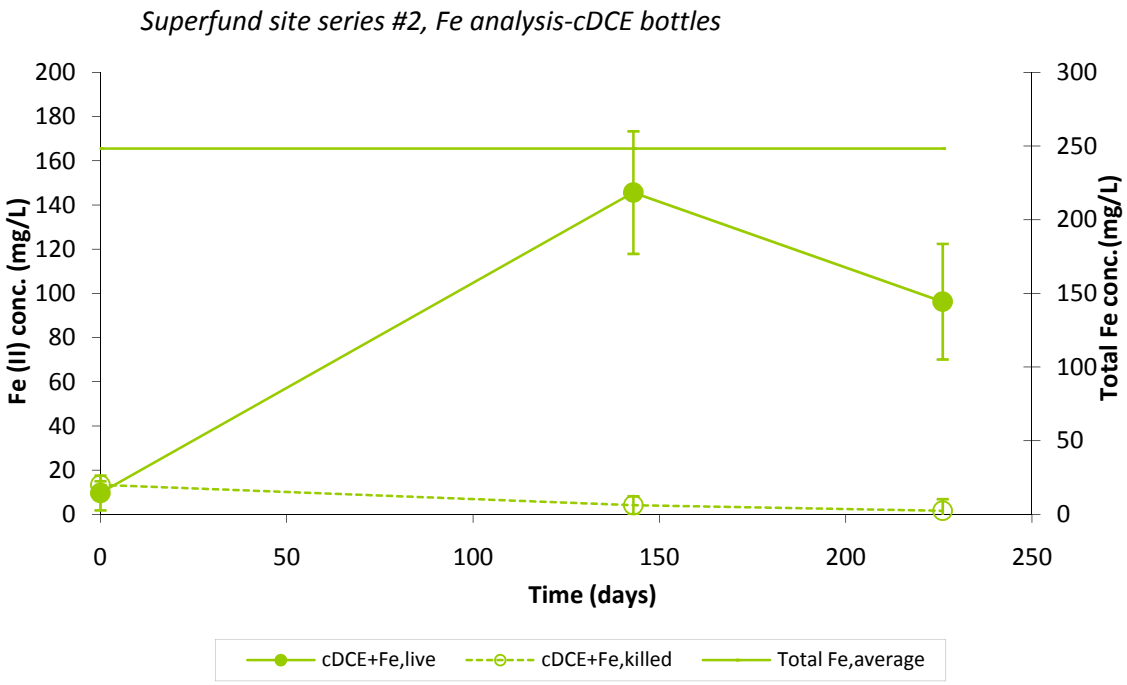


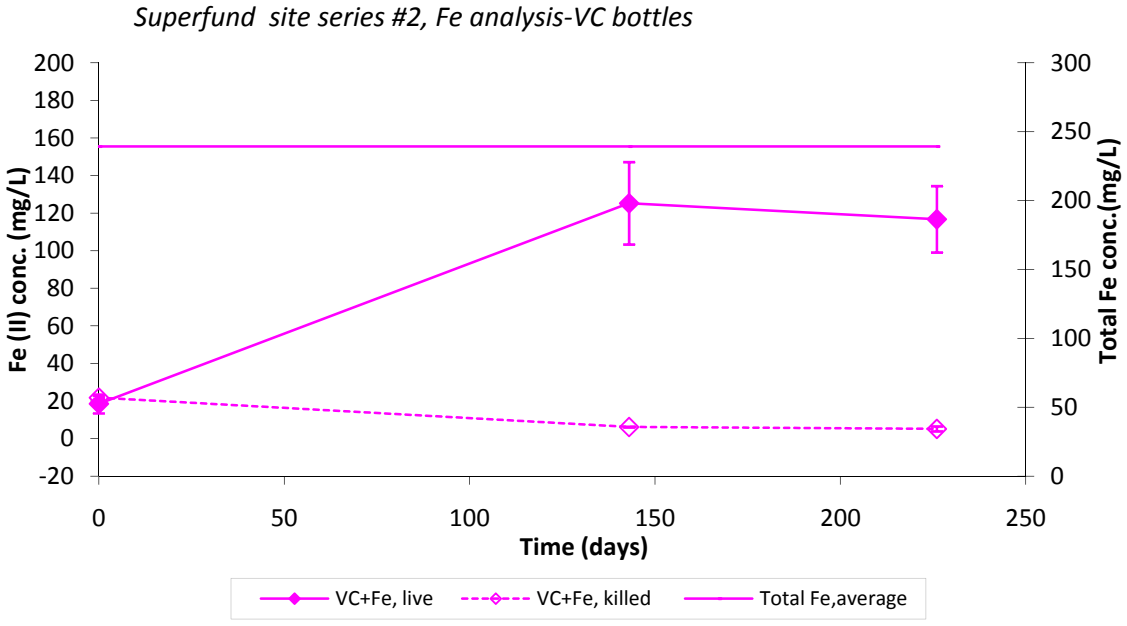

Superfund site series #2, cDCE w/o Fe 1
CONC.(umole/bottle)
Time (days)
cDCE live 1
cDCE killed 1


Superfund site series #2, cDCE w/o Fe 2
CONC.(umole/bottle)
Time (days)
cDCE live 2
VC,cDCE live 2
cDCE killed 2

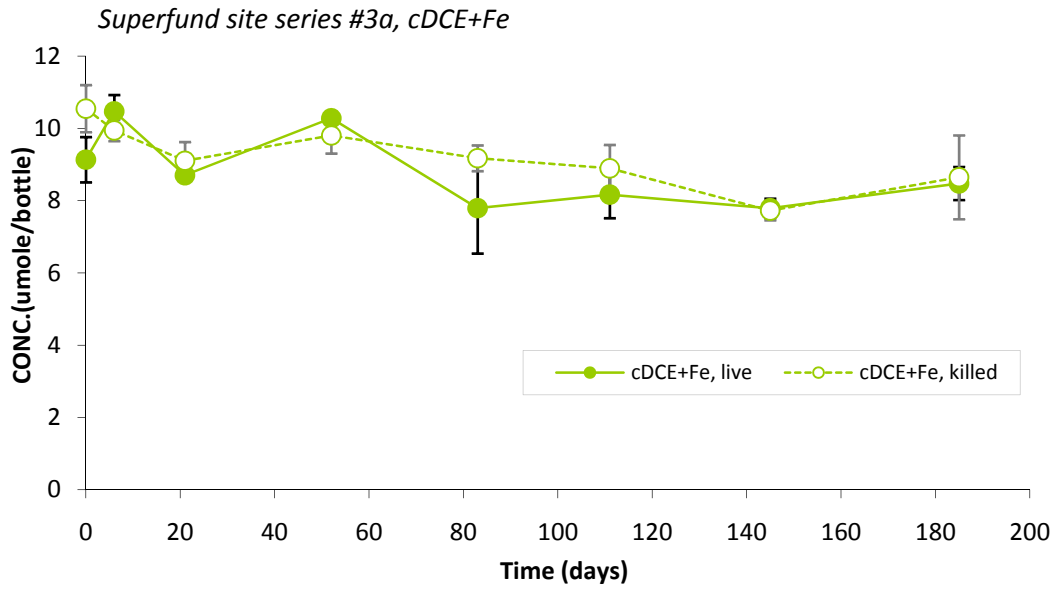

Superfund site series #2,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180

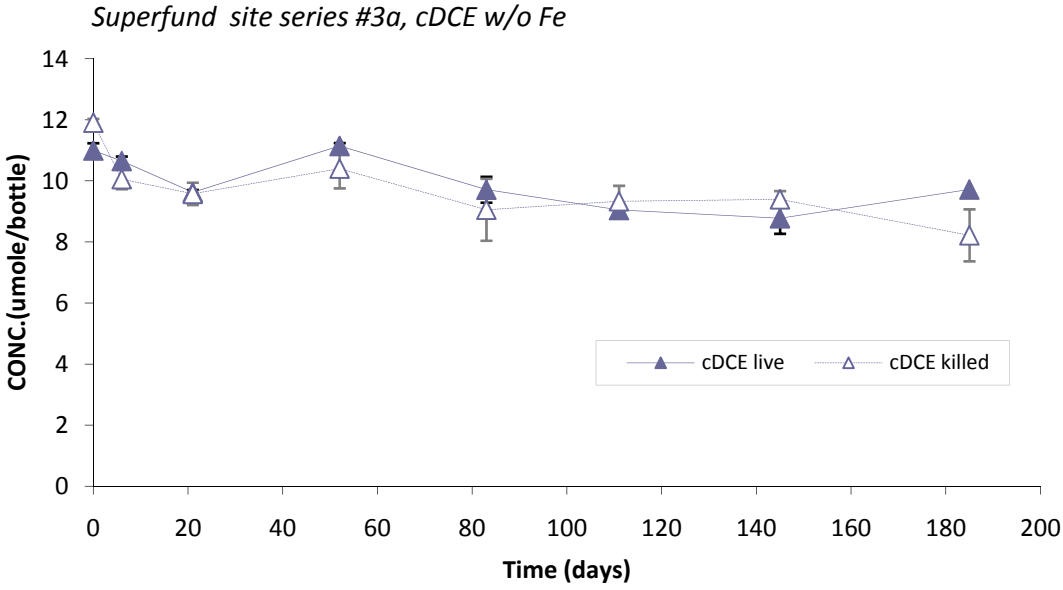

Superfund site series #2,VC w/o Fe
CONC. (umole/bottle)
Time (days)
VC live 1
VC killed 1
ETH,VC live 1
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180

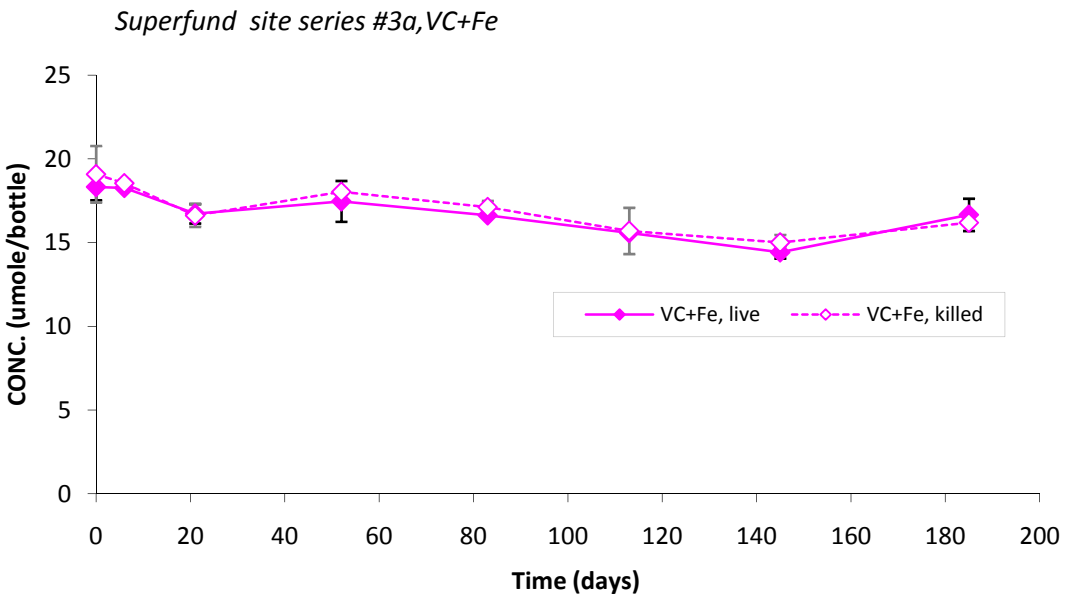

Superfund site series #2, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

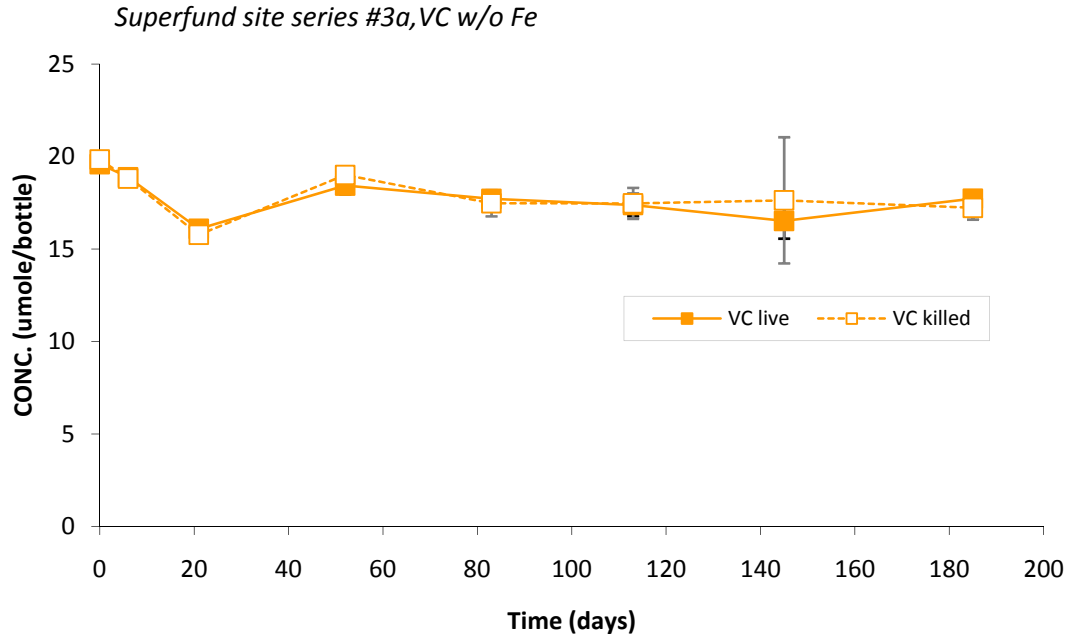

Superfund site series #2, Fe analysis-VC bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
VC+Fe, live
VC+Fe, killed
Total Fe,average

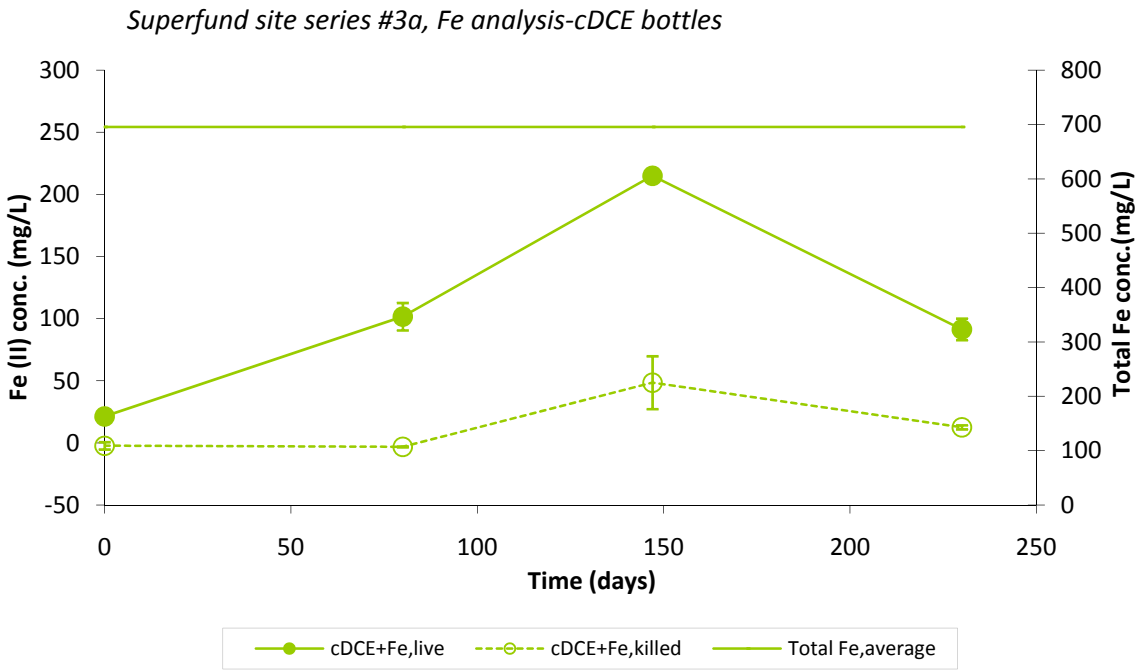
6-C. Site/Source 6: Undisclosed Superfund Site Series #3-(a):

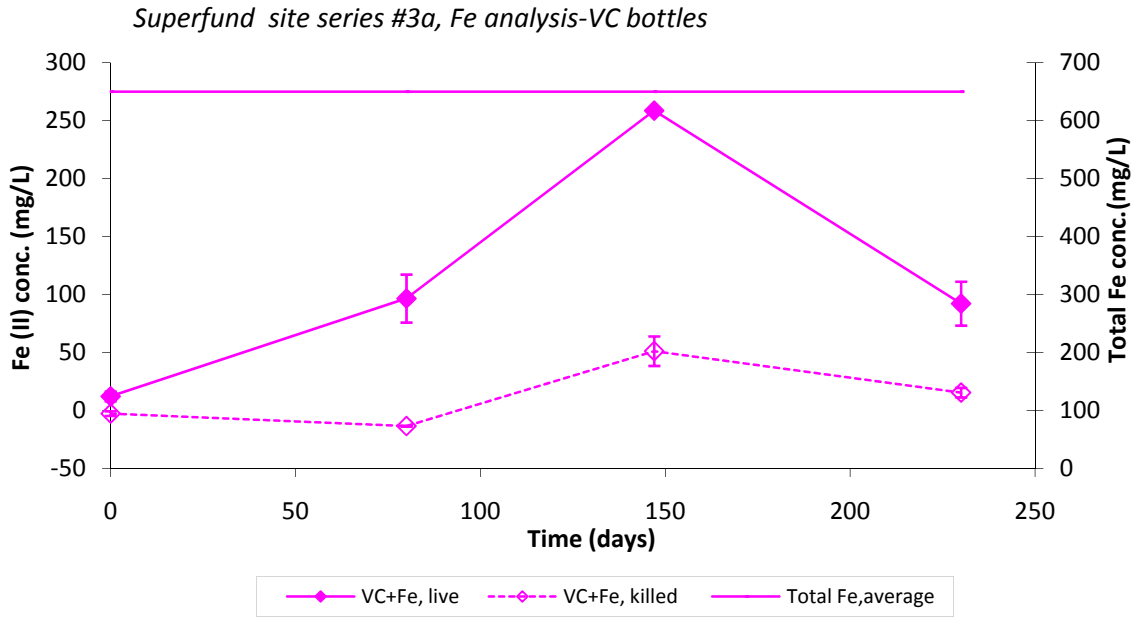




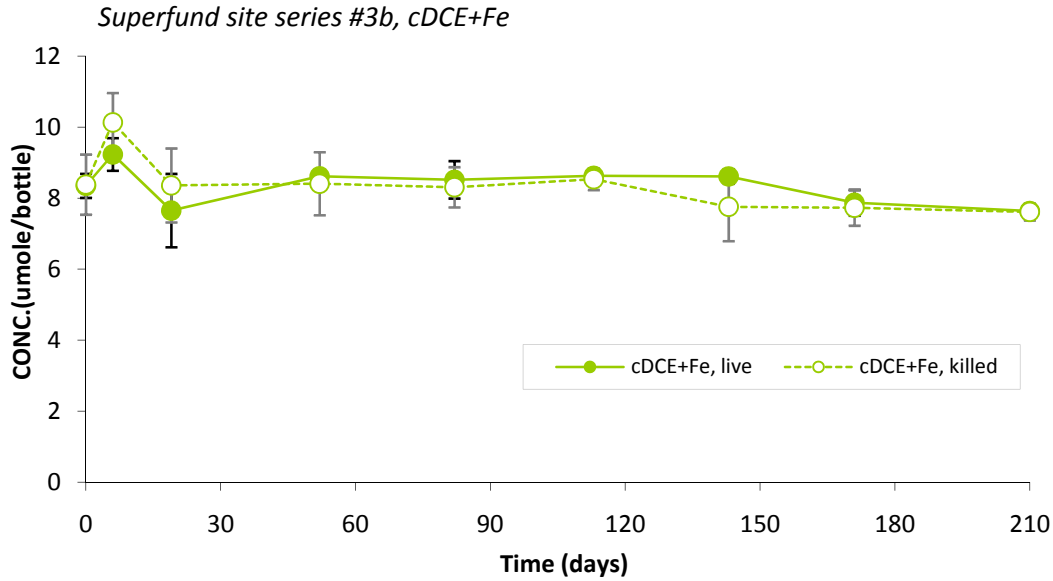

Superfund site series #3a,VC+Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
180
200
Time (days)
VC+Fe, live
VC+Fe, killed

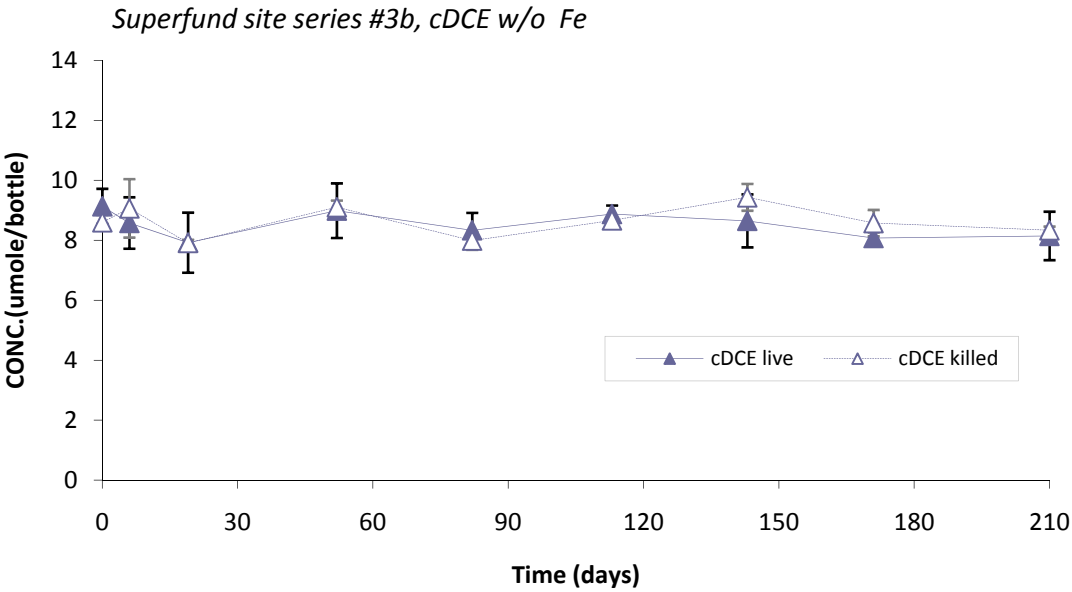

Superfund site series #3a,VC w/o Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
180
200
Time (days)
VC live
VC killed

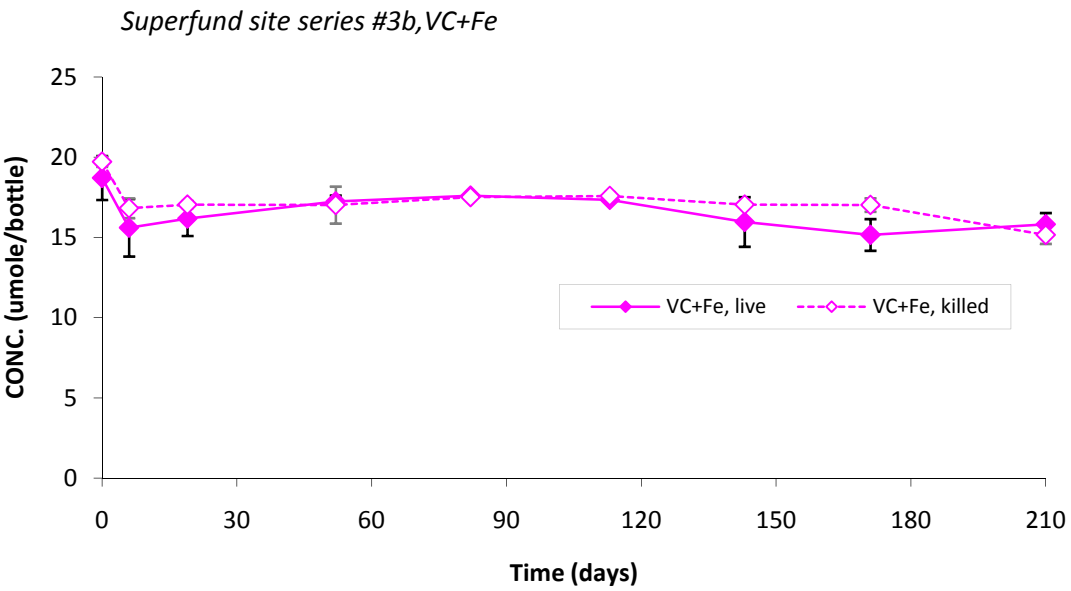

Superfund site series #3a, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

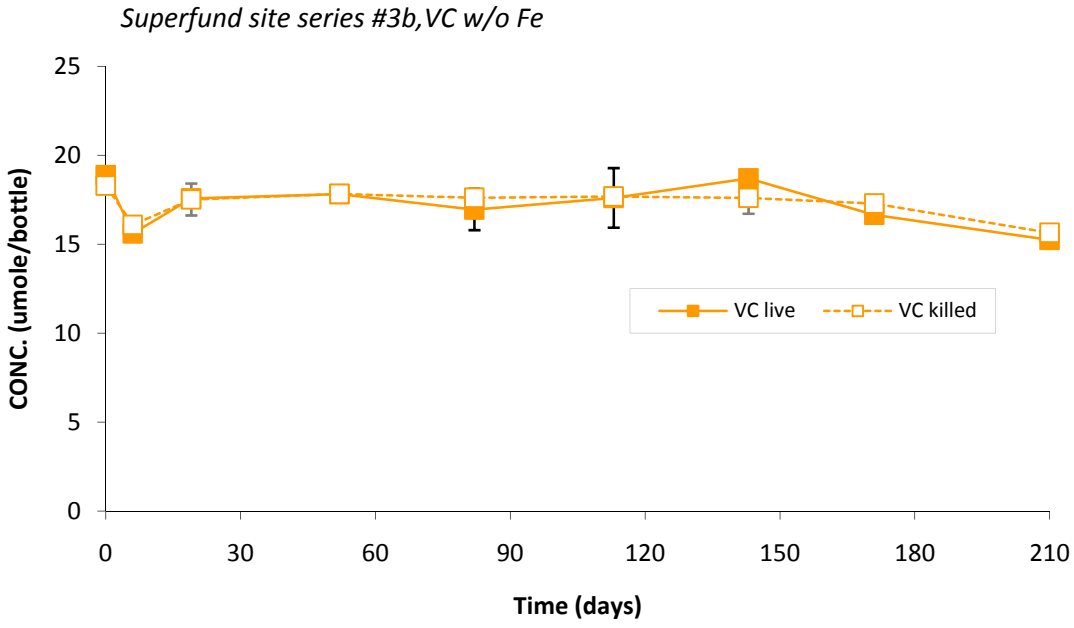

Superfund site series #3a, Fe analysis-VC bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
VC+Fe, live
VC+Fe, killed
Total Fe,average

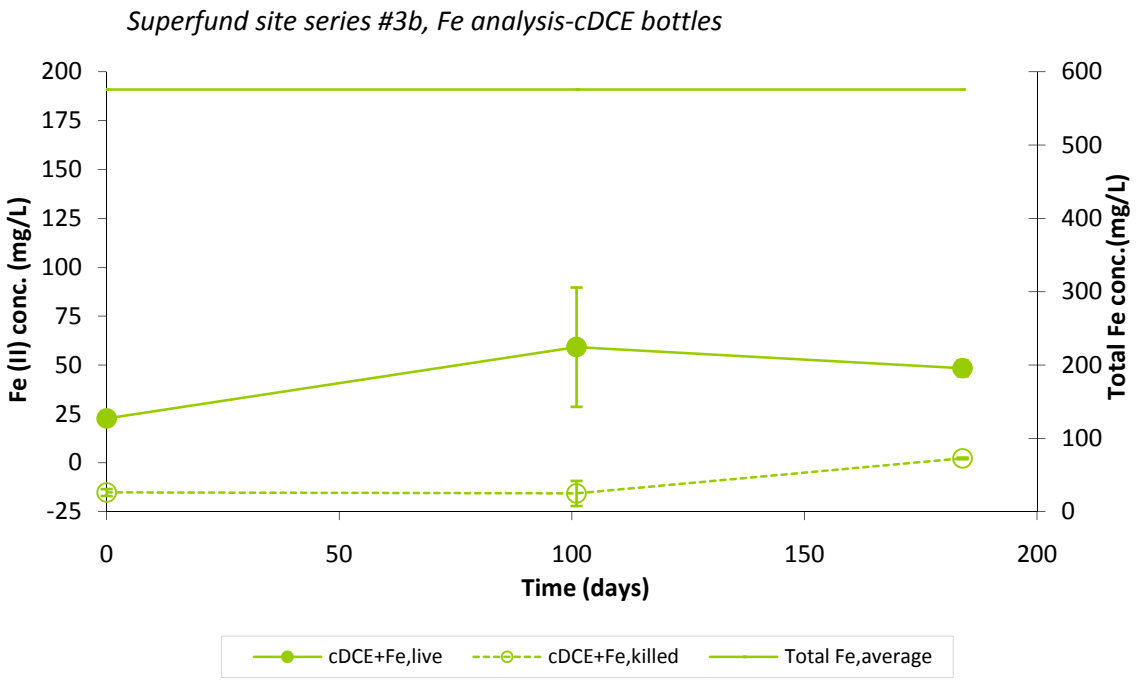
6-D. Site/Source 6: Undisclosed Superfund Site Series #3-(b):

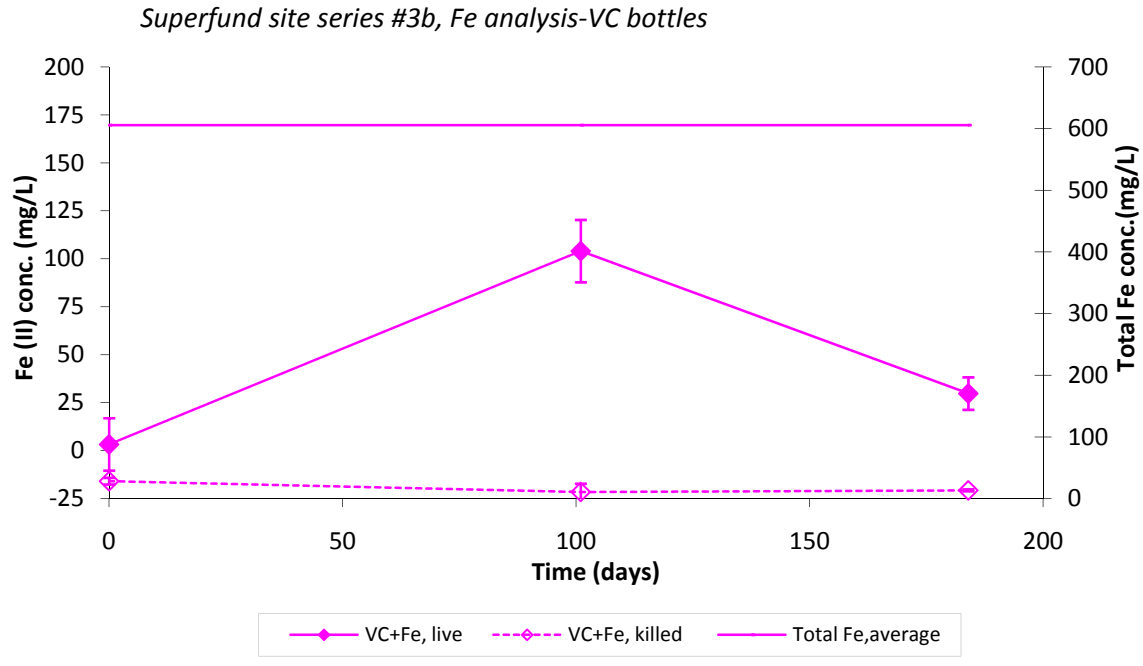




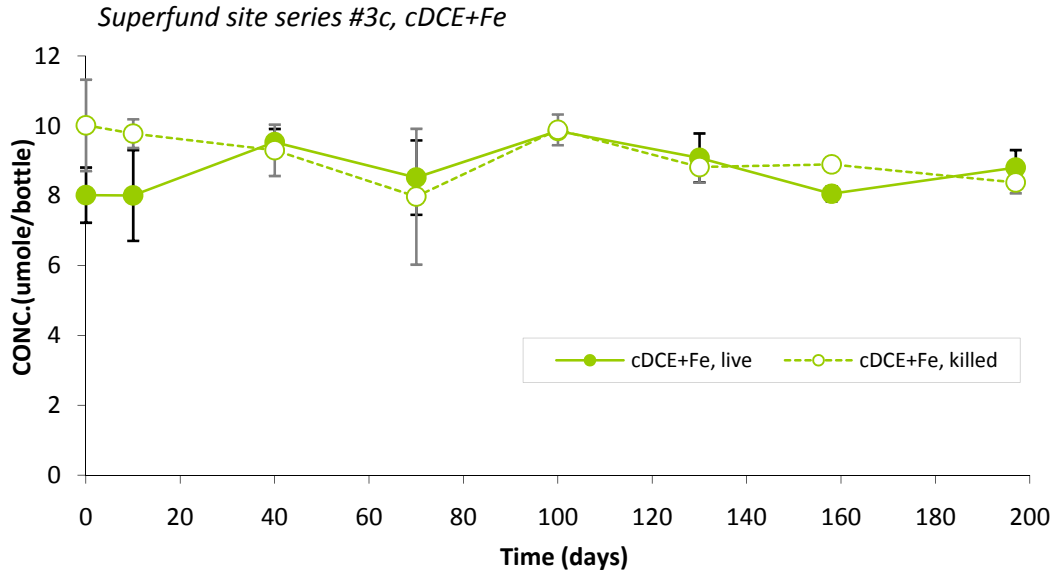

Superfund site series #3b,VC+Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
30
60
90
120
150
180
210
Time (days)
VC+Fe, live
VC+Fe, killed

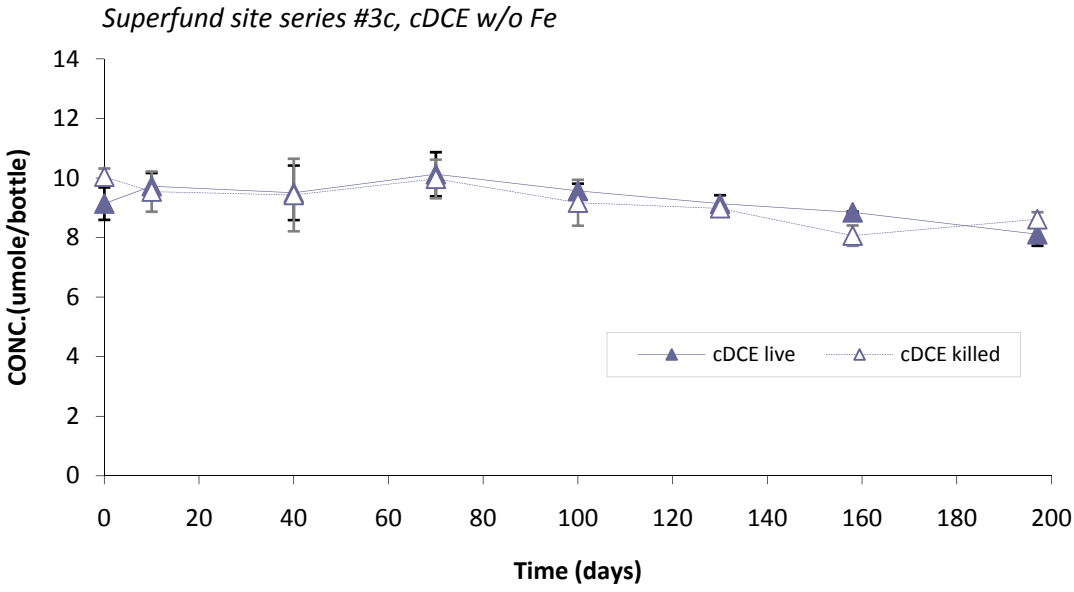

Superfund site series #3b,VC w/o Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
30
60
90
120
150
180
210
Time (days)
VC live
VC killed

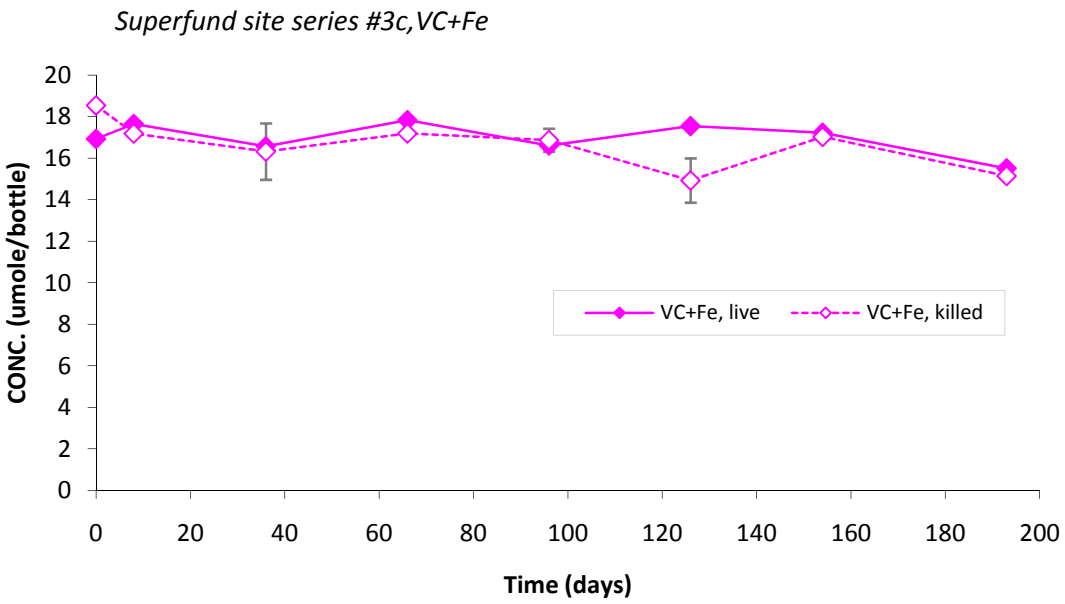

Superfund site series #3b, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

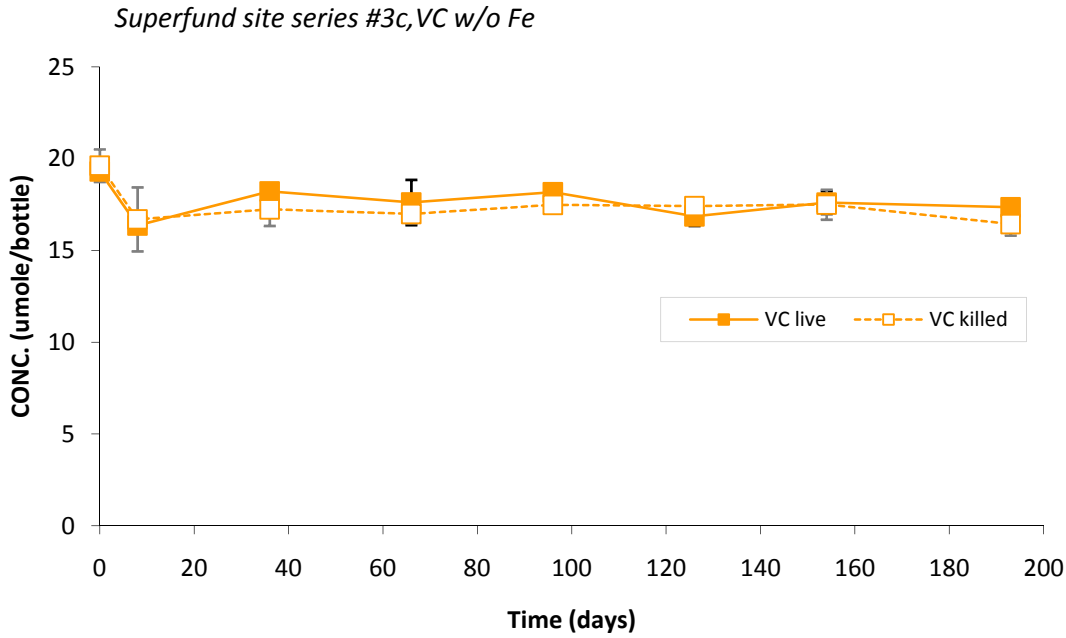

Superfund site series #3b, Fe analysis-VC bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
VC+Fe, live
VC+Fe, killed
Total Fe,average

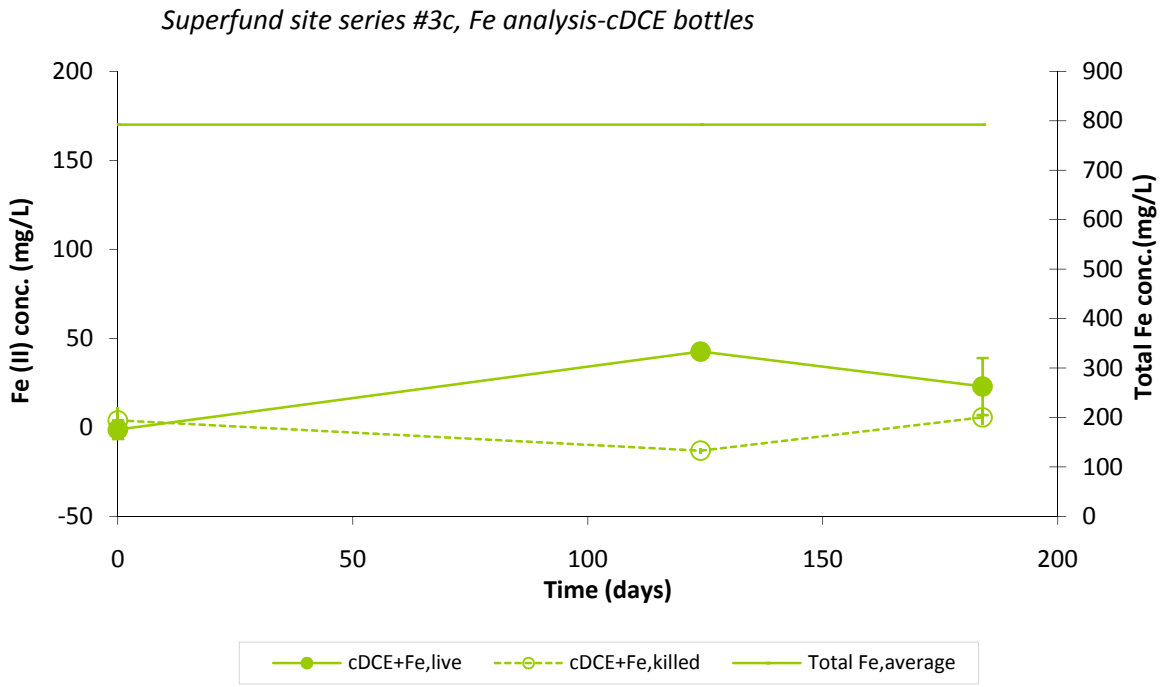
6-E. Site/Source 6: Undisclosed Superfund Site Series #3-(c):

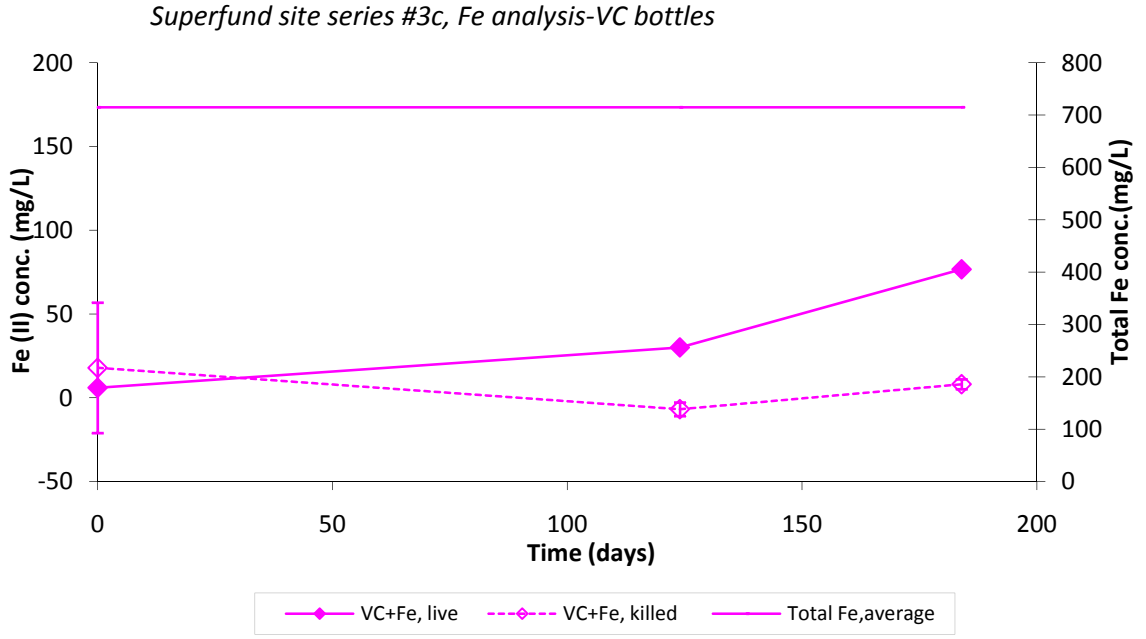




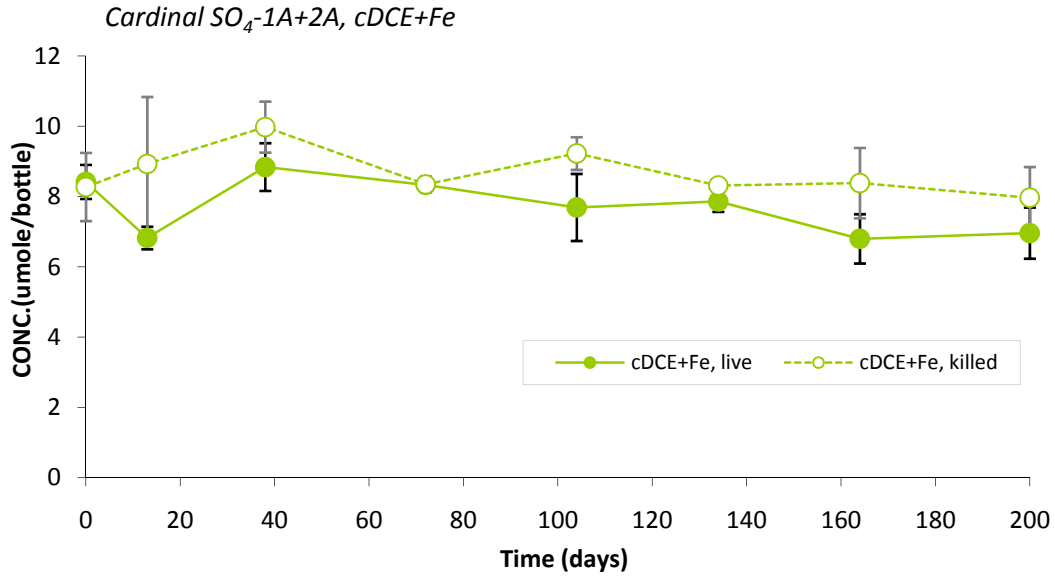

Superfund site series #3c,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed

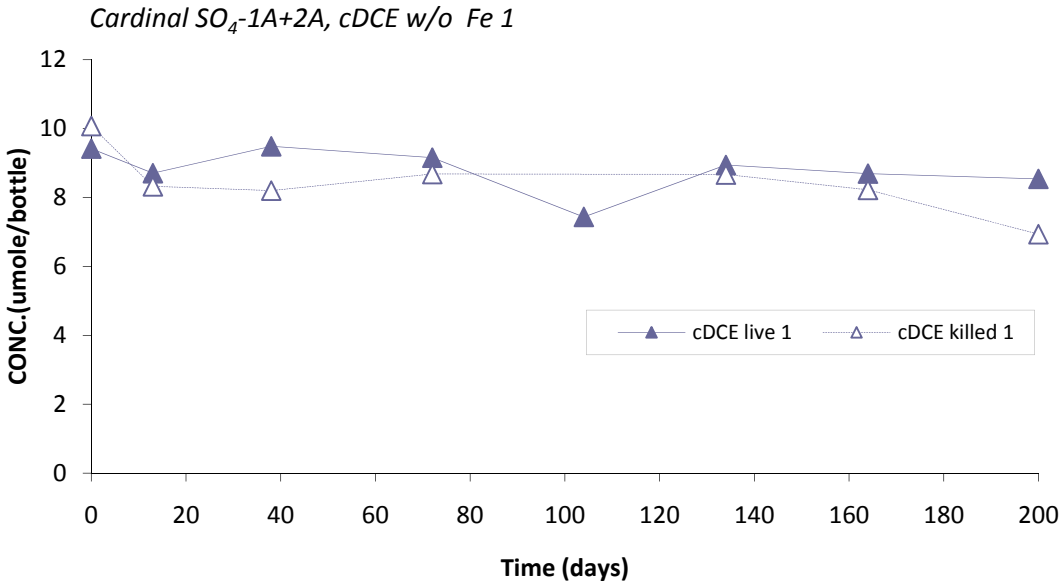

Superfund site series #3c,VC w/o Fe
CONC. (umole/bottle)
Time (days)
VC live
VC killed

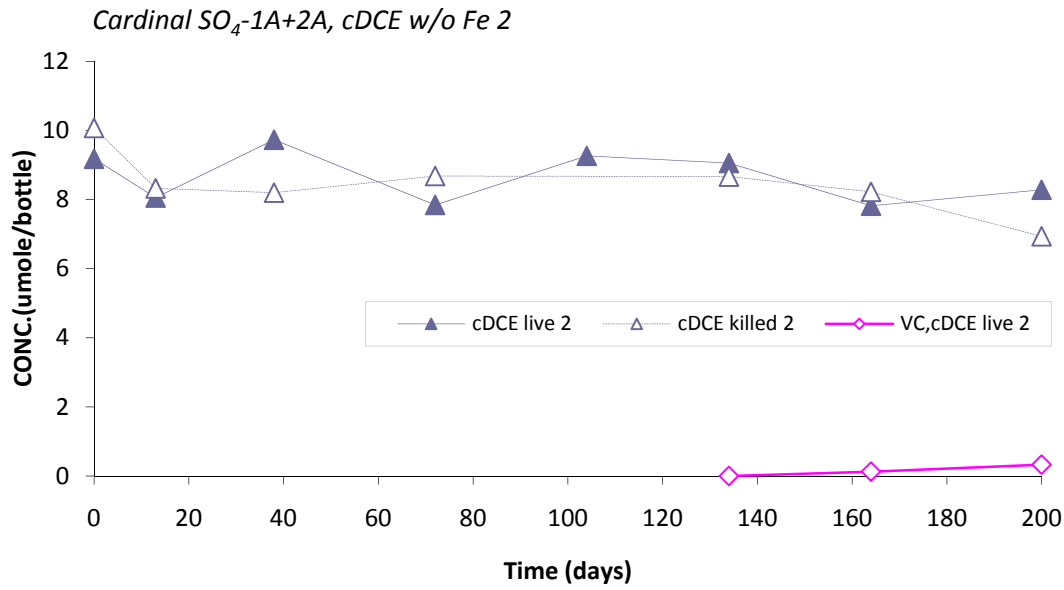

Superfund site series #3c, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

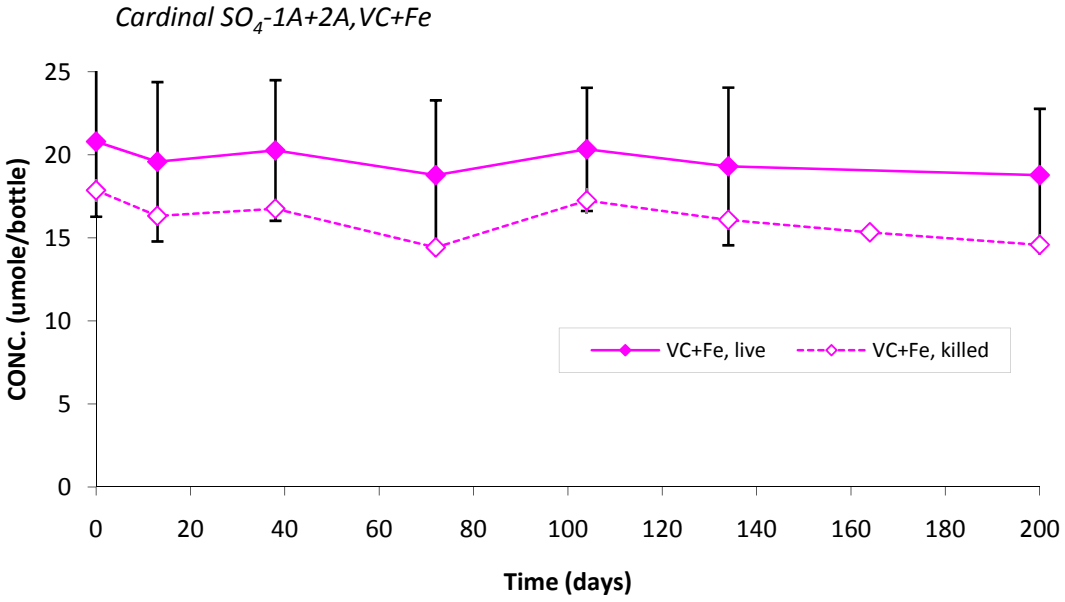

Superfund site series #3c, Fe analysis-VC bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
VC+Fe, live
VC+Fe, killed
Total Fe,average

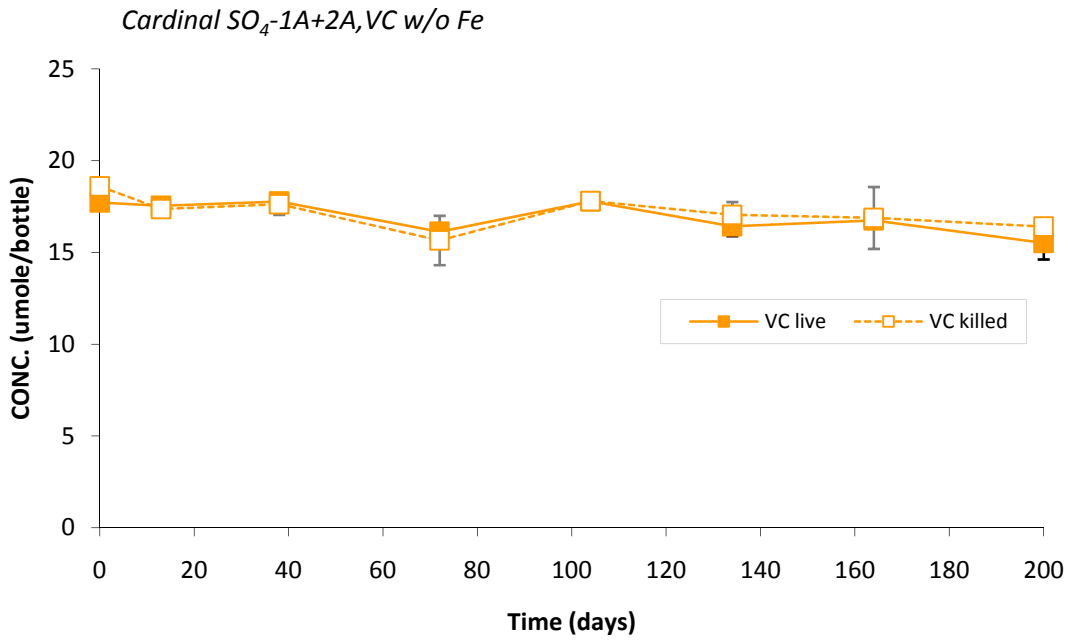
7-A. Site/Source 7: Cardinal Landfill SO_4-1A + SO_4-2A:

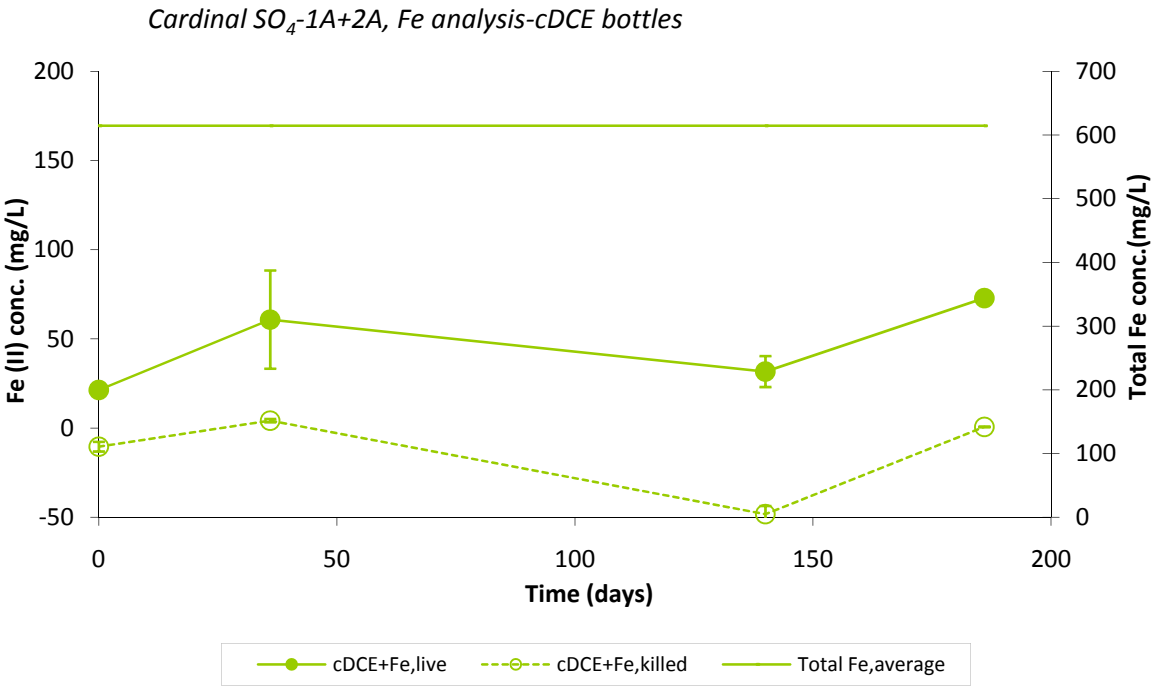


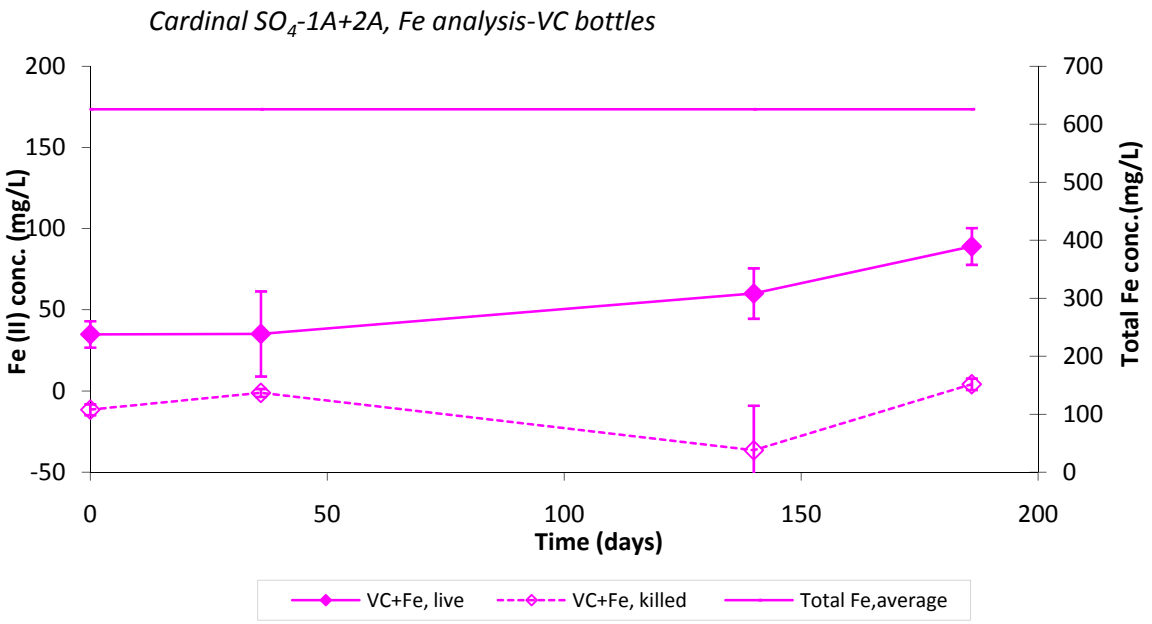



Cardinal SO4-1A+2A, cDCE w/o Fe 2
CONC.(umole/bottle)
Time (days)
cDCE live 2
cDCE killed 2
VC,cDCE live 2
0
2
4
6
8
10
12
0
20
40
60
80
100
120
140
160
180
200

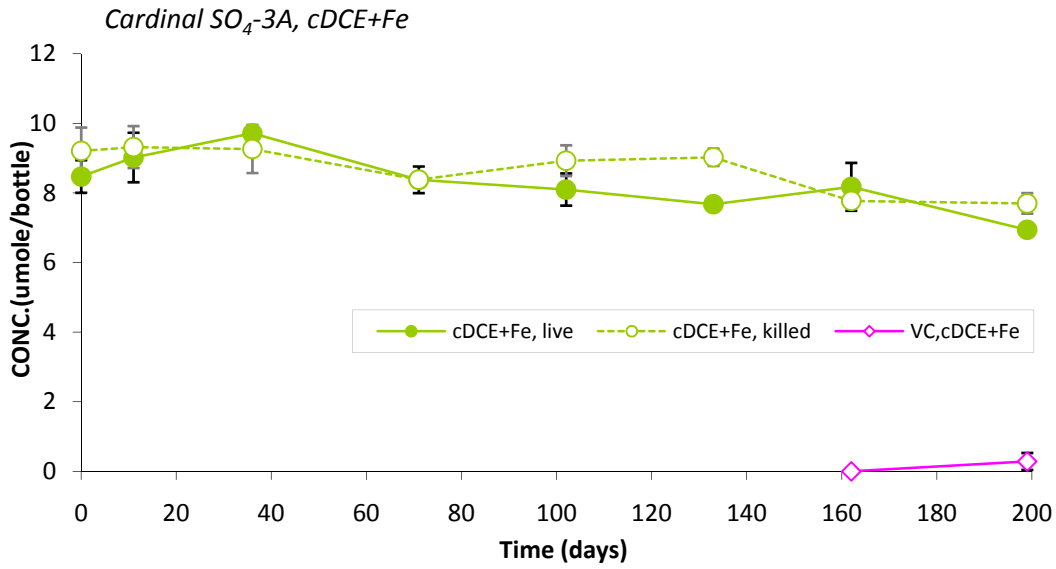

Cardinal SO4-1A+2A,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180
200

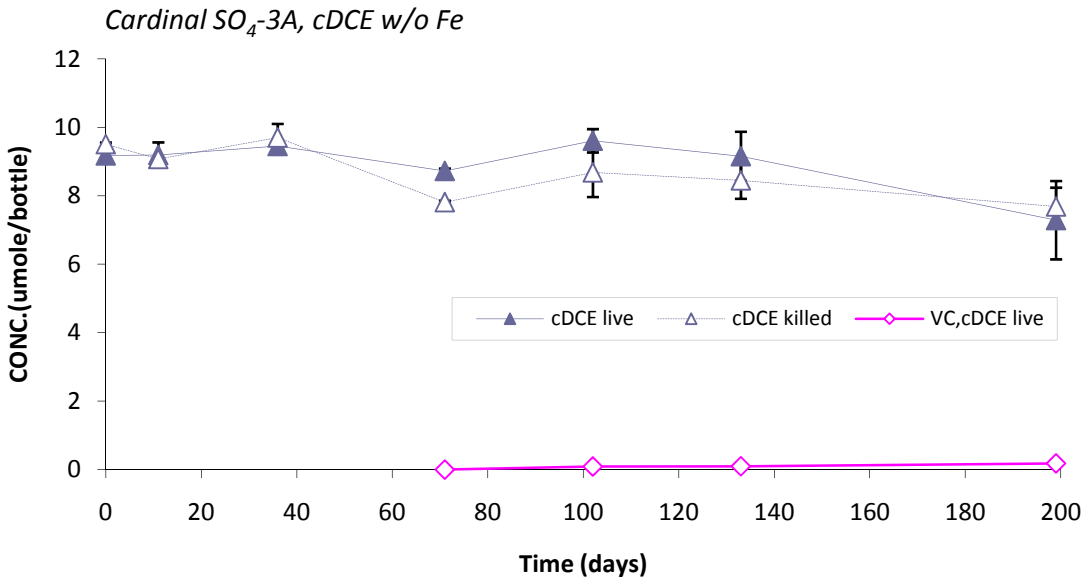

Cardinal SO4-1A+2A,VC w/o Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
180
200
Time (days)
VC live
VC killed

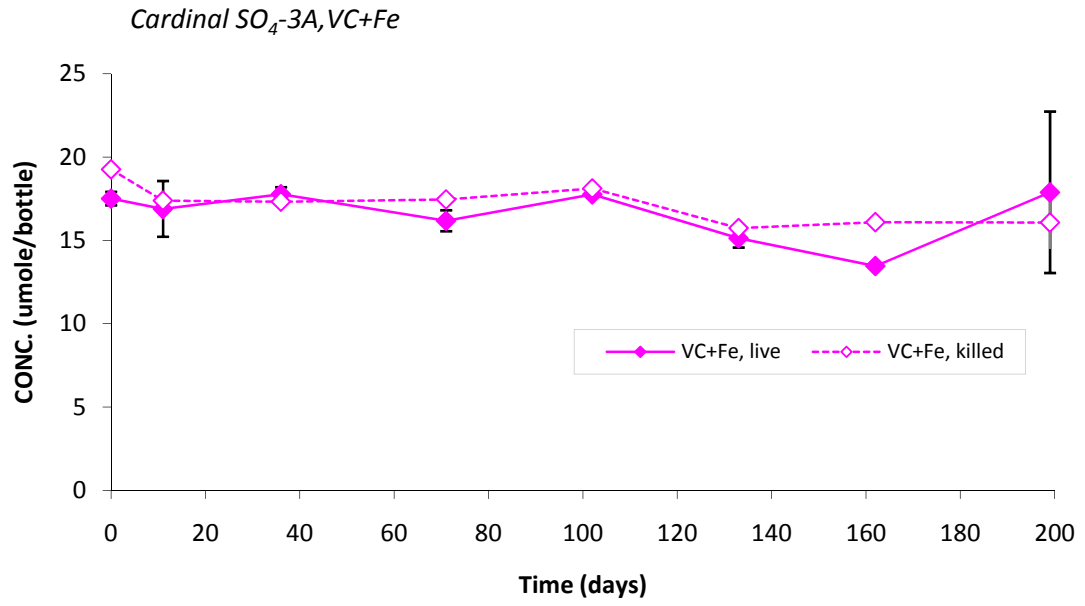

Cardinal SO4-1A+2A, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
200
150
100
50
0
-50
Total Fe conc.(mg/L)
700
600
500
400
300
200
100
0
0
50
100
150
200
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

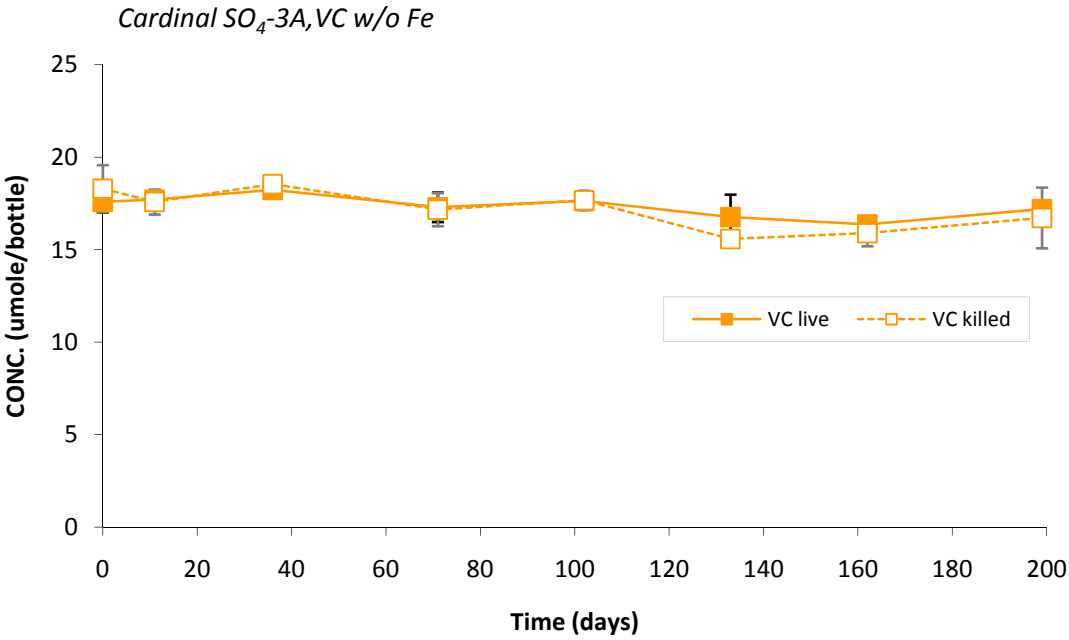


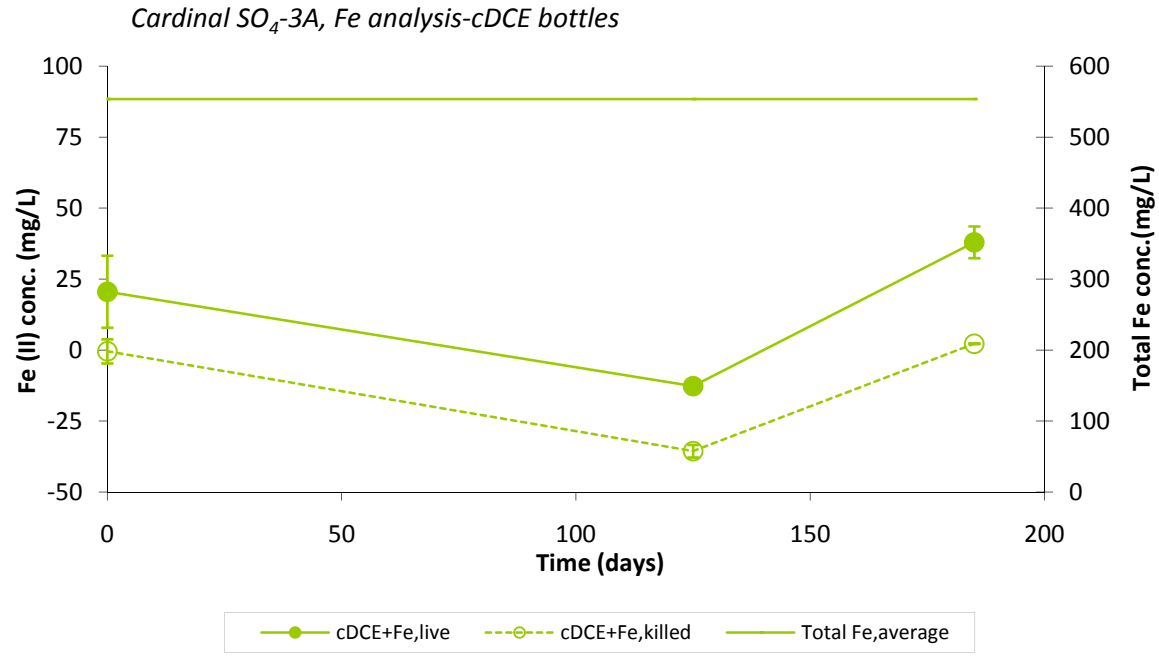
7-B. Site/Source 7: Cardinal Landfill SO_4-3A:

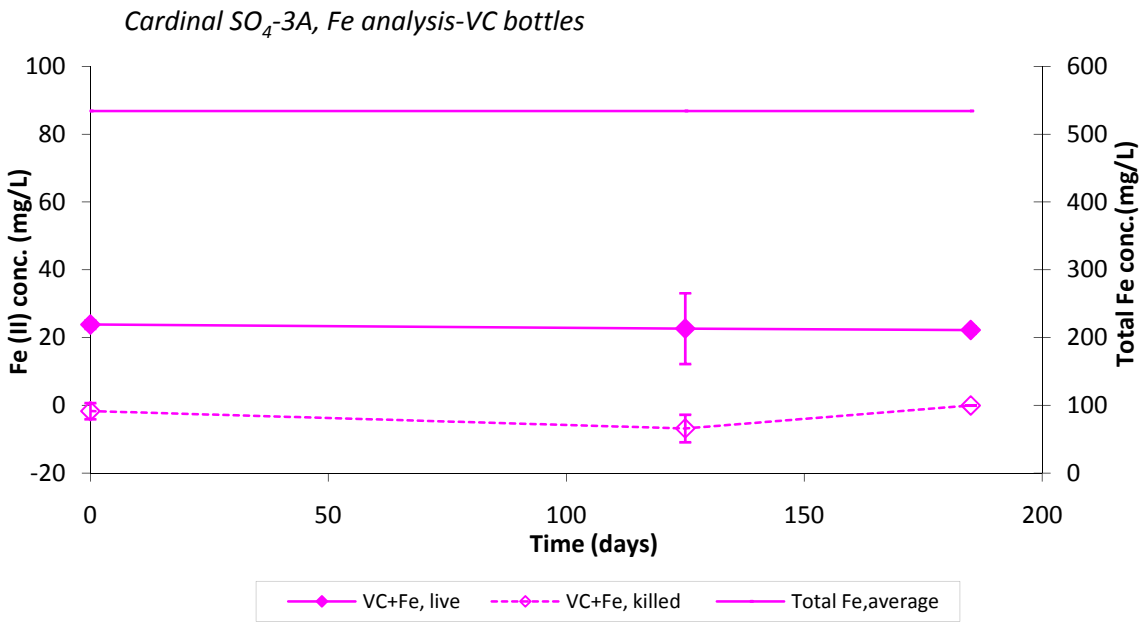



Cardinal SO4-3A, cDCE w/o Fe
CONC.(umole/bottle)
Time (days)
cDCE live
cDCE killed
VC,cDCE live
0
2
4
6
8
10
12
0
20
40
60
80
100
120
140
160
180
200

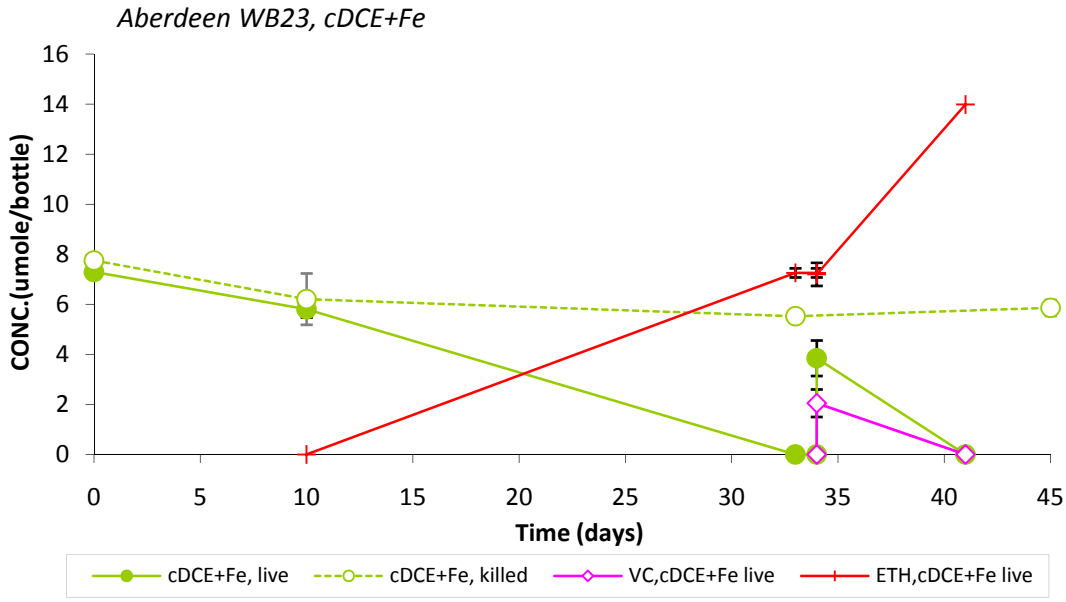

Cardinal SO4-3A,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180
200

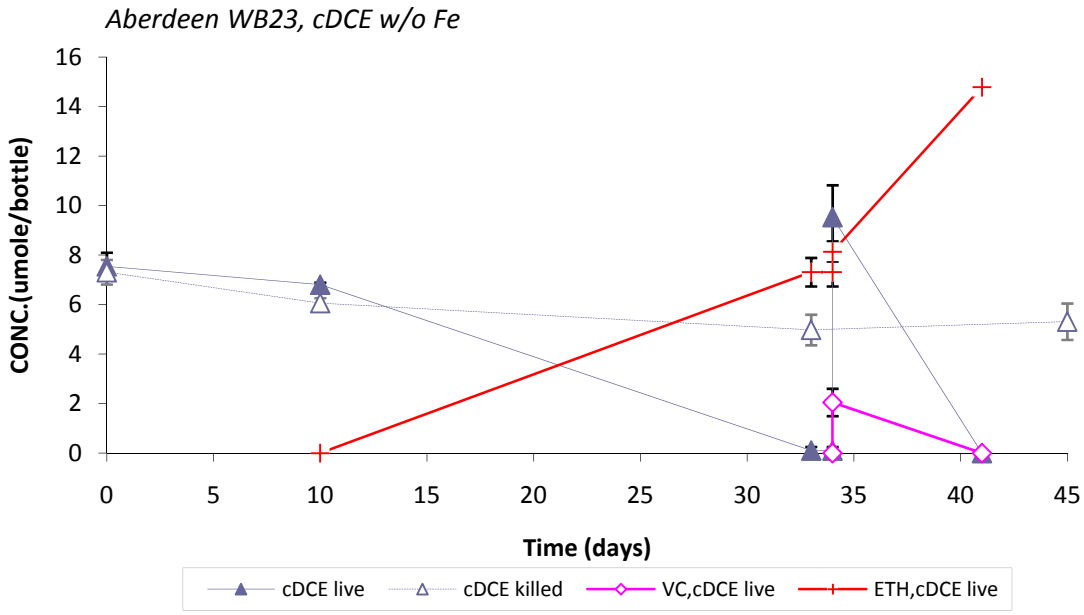

Cardinal SO4-3A,VC w/o Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
180
200
Time (days)
VC live
VC killed

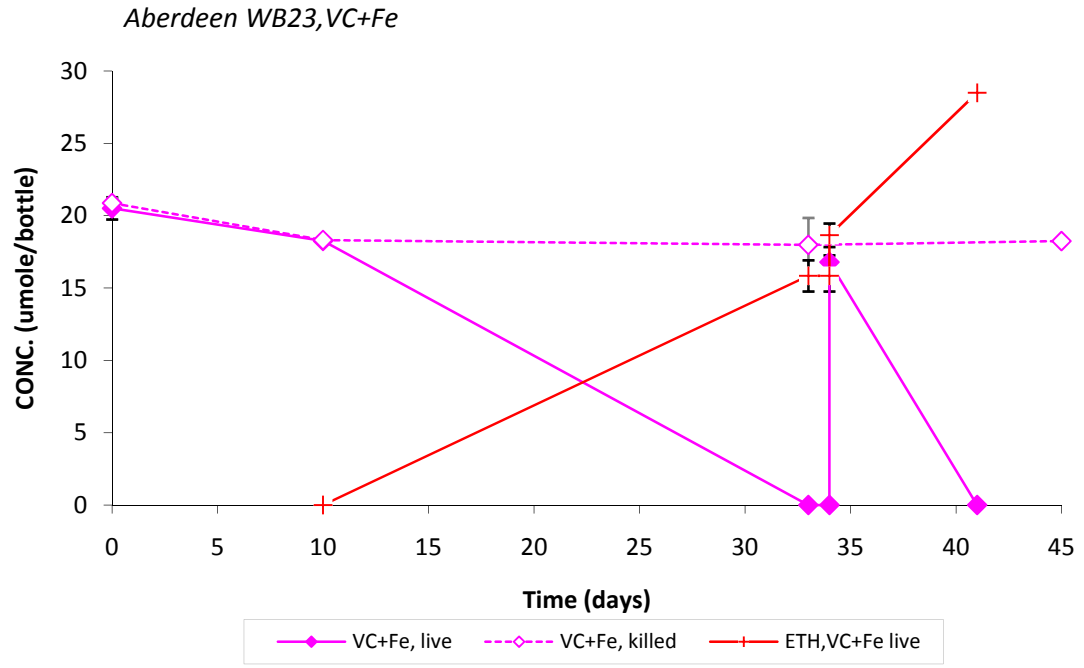

Cardinal SO4-3A, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
100
75
50
25
0
-25
-50
Total Fe conc.(mg/L)
600
500
400
300
200
100
0
0
50
100
150
200
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

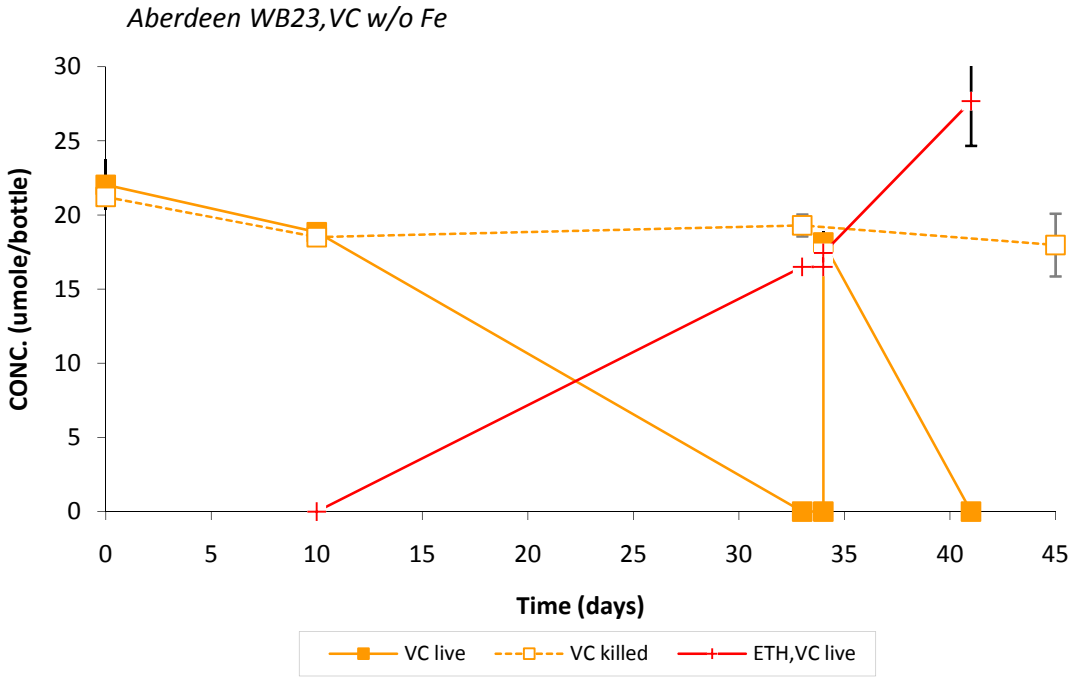


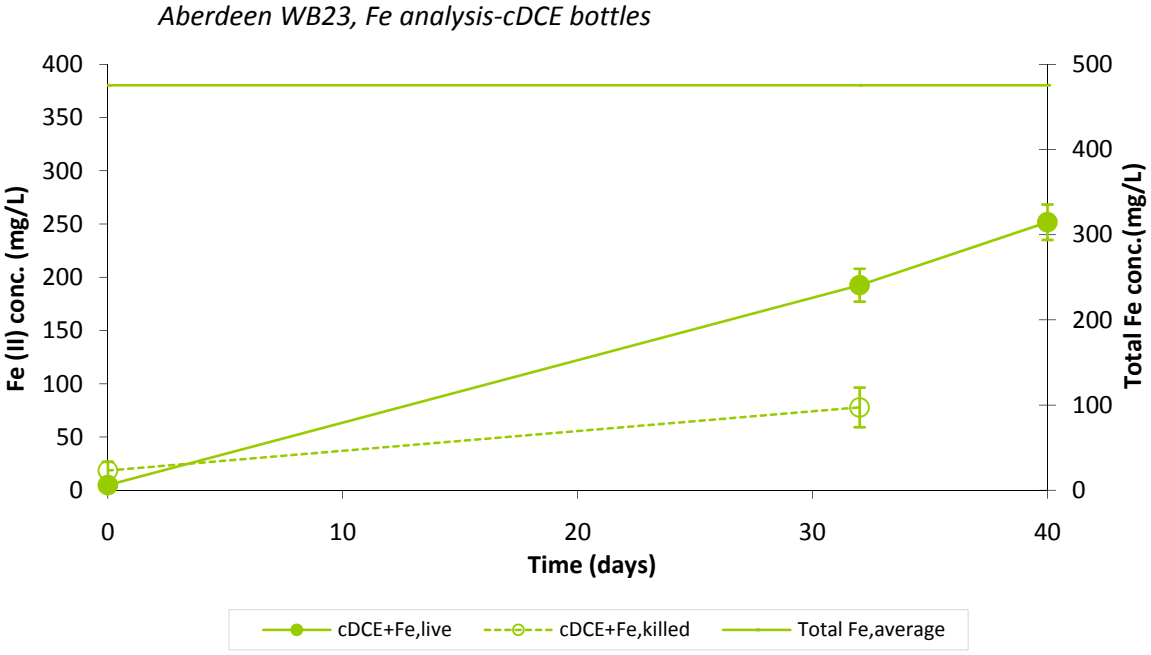
8-A. Site/Source 8: Aberdeen Proving Ground WB23:

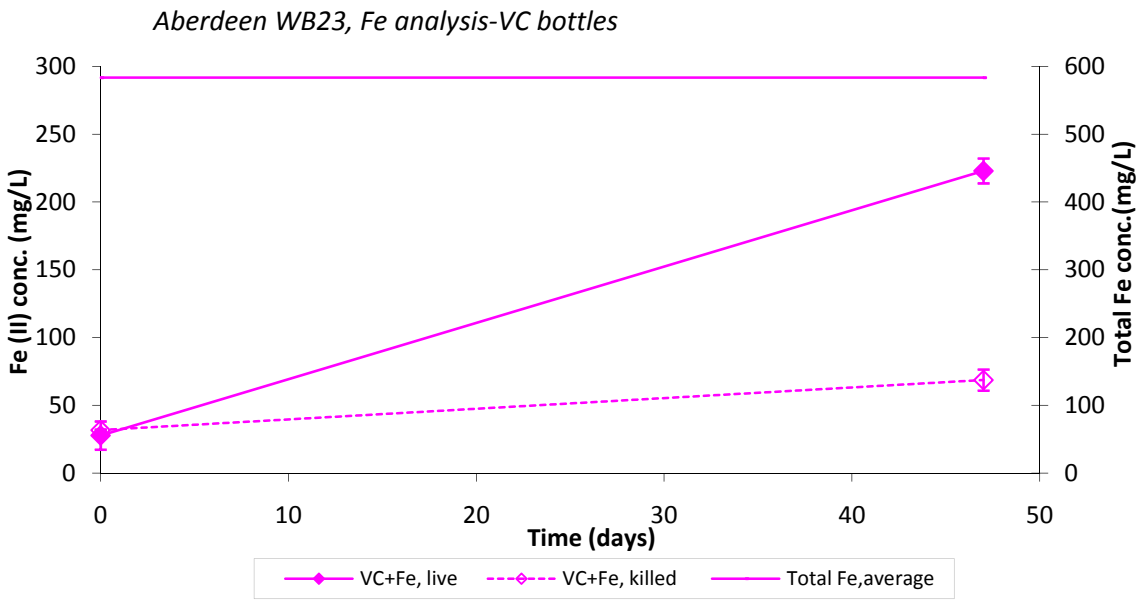



Aberdeen WB23, cDCE w/o Fe
CONC.(umole/bottle)
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
45
Time (days)
cDCE live
cDCE killed
VC,cDCE live
ETH,cDCE live

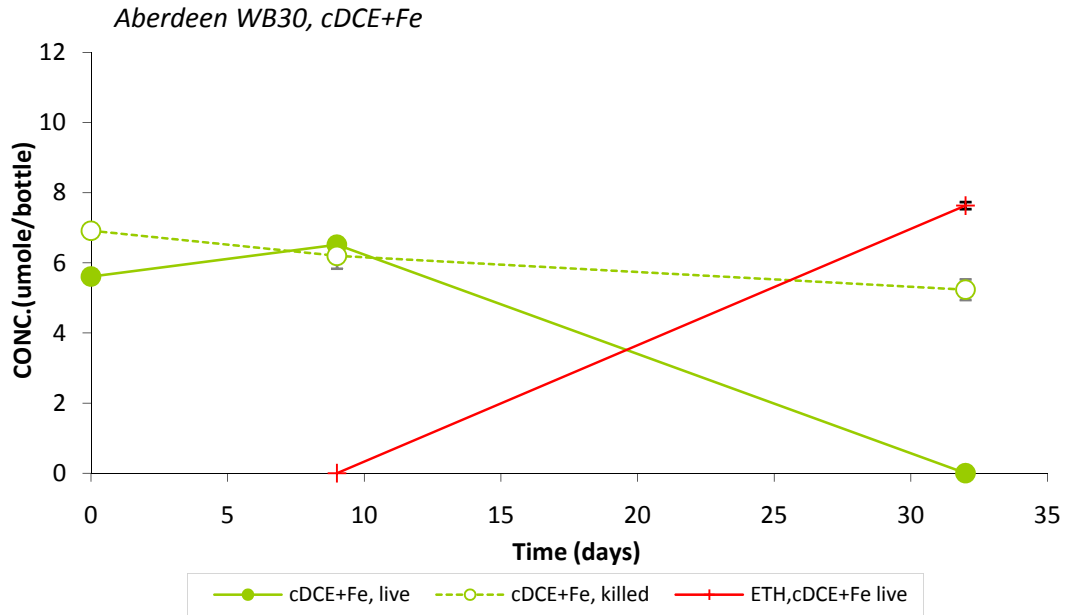

Aberdeen WB23,VC+Fe
CONC. (umole/bottle)
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
45
Time (days)
VC+Fe, live
VC+Fe, killed
ETH,VC+Fe live

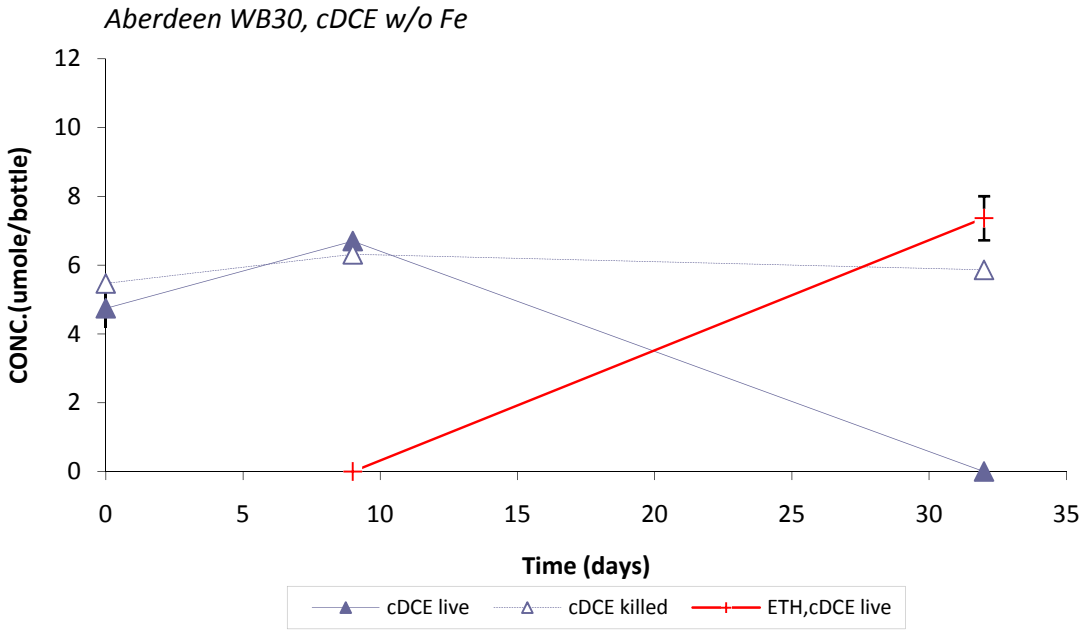

Aberdeen WB23,VC w/o Fe
CONC. (umole/bottle)
Time (days)
VC live
VC killed
ETH,VC live

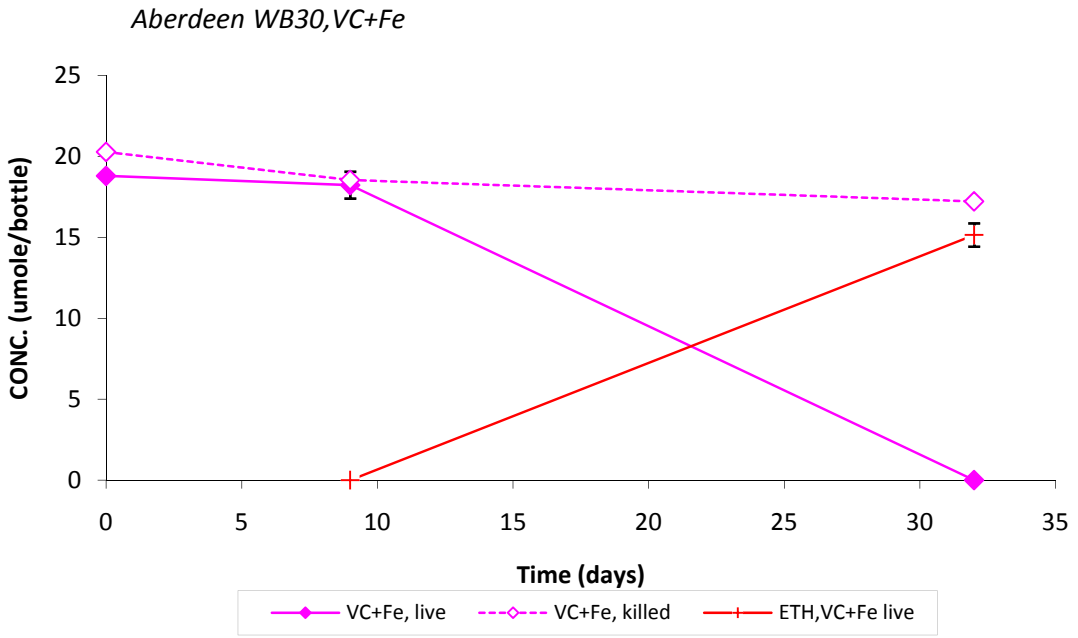

Aberdeen WB23, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
Total Fe conc.(mg/L)
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

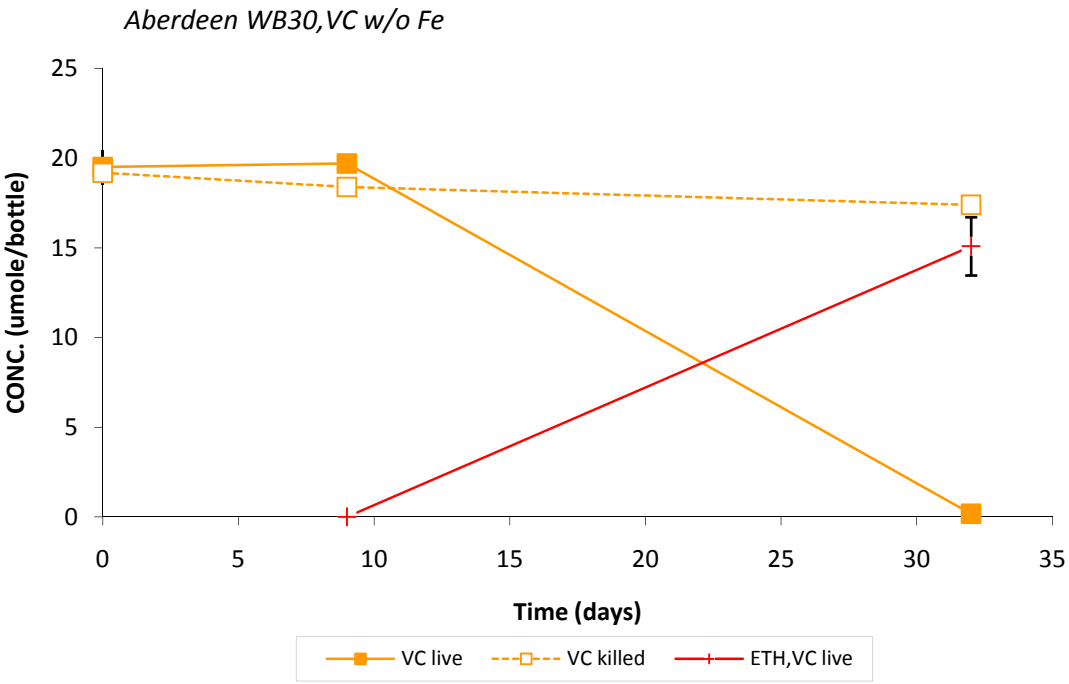


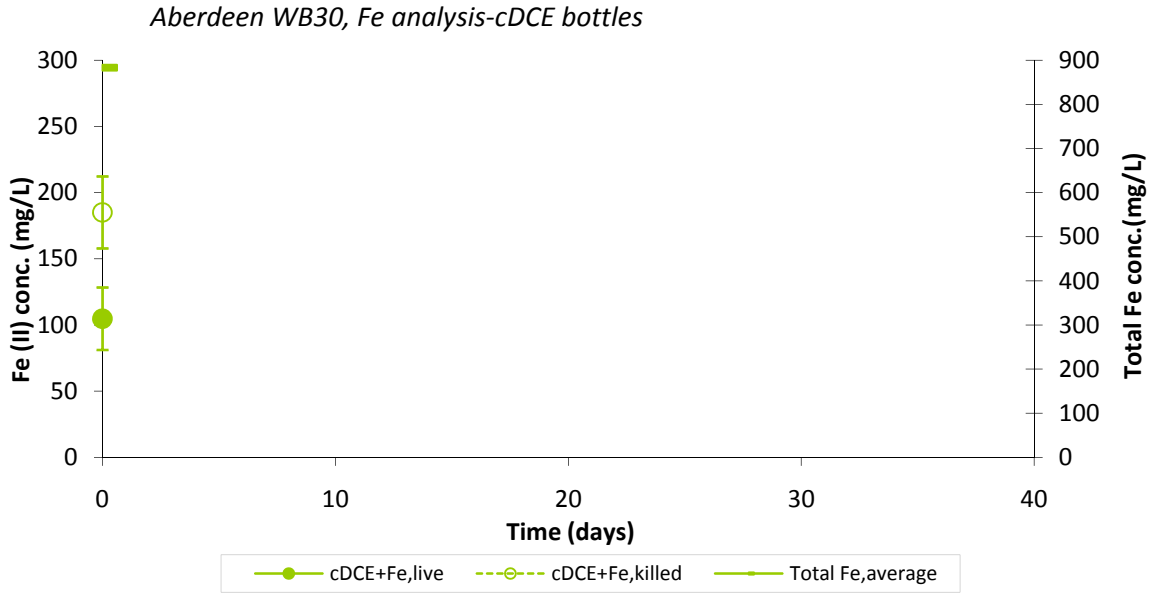
8-B. Site/Source 8: Aberdeen Proving Ground WB30:

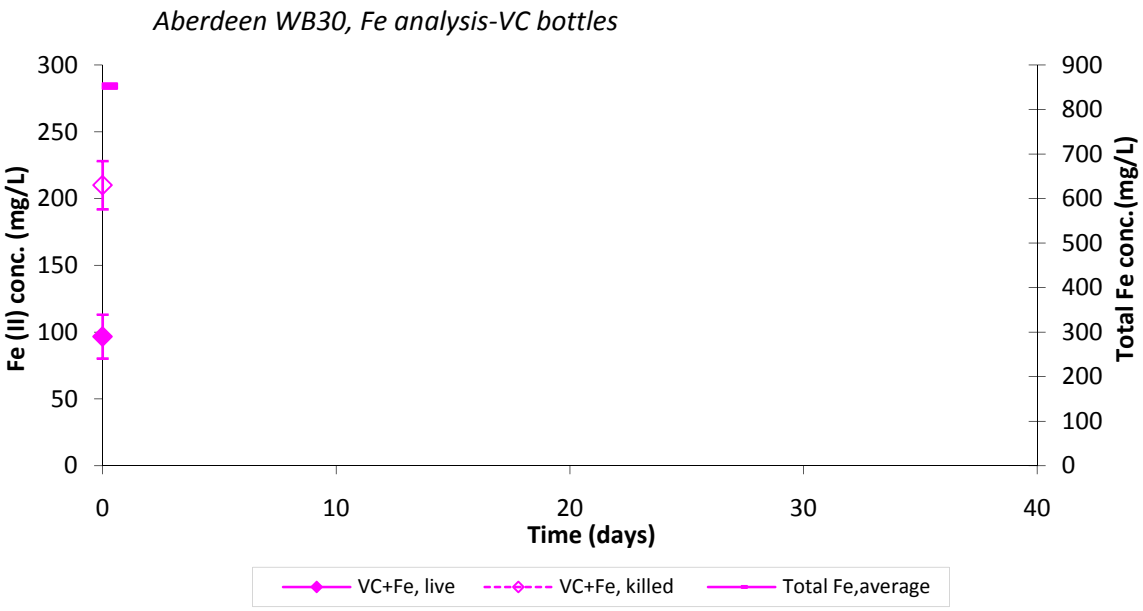



Aberdeen WB30, cDCE w/o Fe
CONC.(umole/bottle)
Time (days)
cDCE live
cDCE killed
ETH,cDCE live

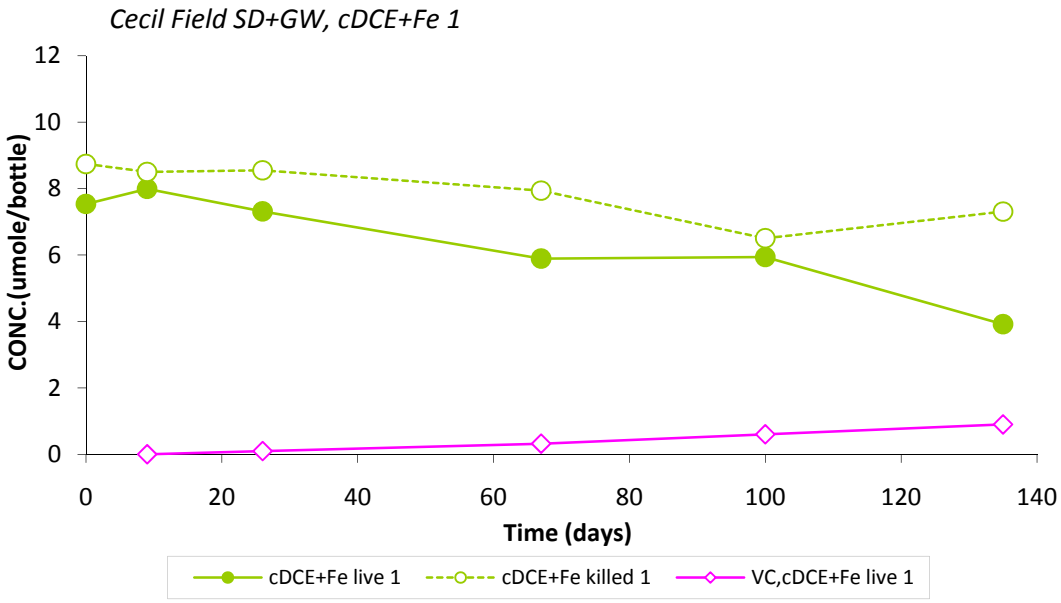

Aberdeen WB30,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe, live
VC+Fe, killed
ETH,VC+Fe live

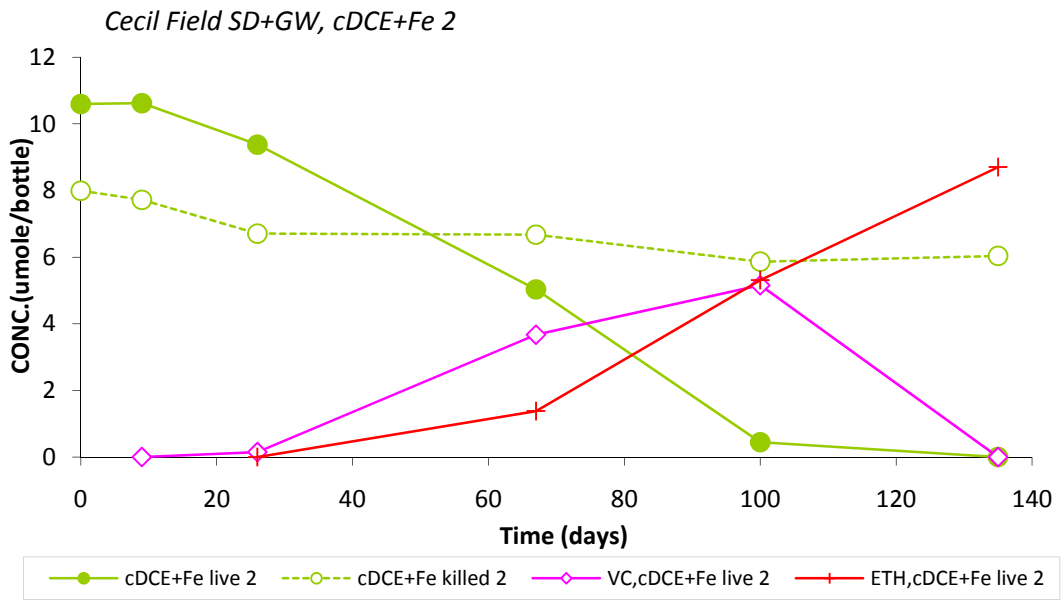

Aberdeen WB30,VC w/o Fe
CONC. (umole/bottle)
25
20
15
10
5
0
0
5
10
15
20
25
30
35
Time (days)
VC live
VC killed
ETH,VC live

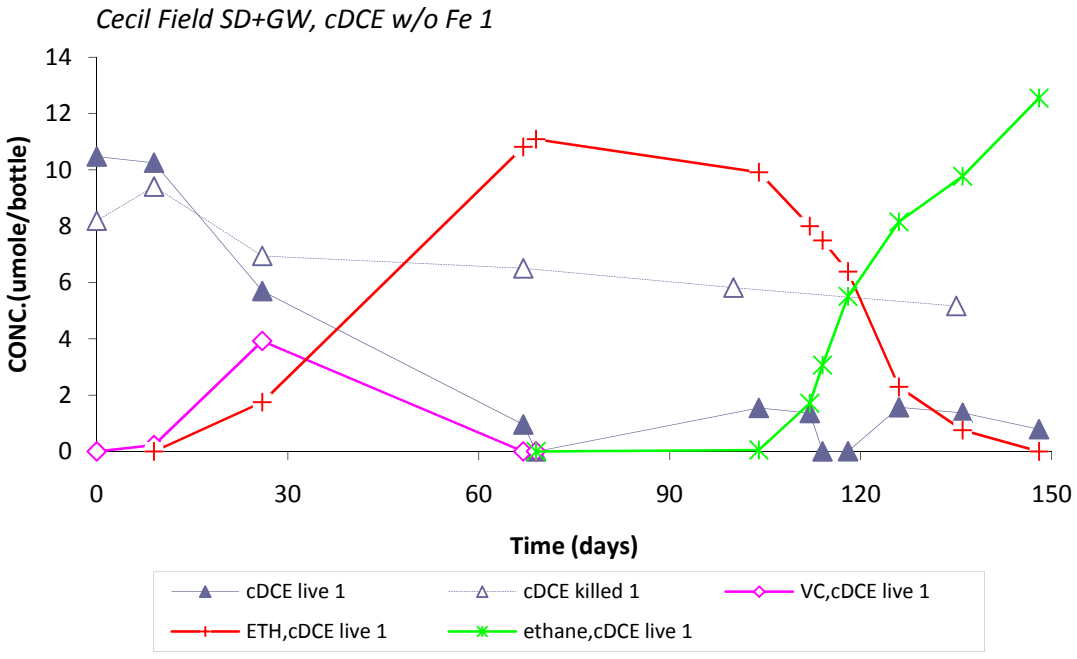

Aberdeen WB30, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
300
250
200
150
100
50
0
Total Fe conc.(mg/L)
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

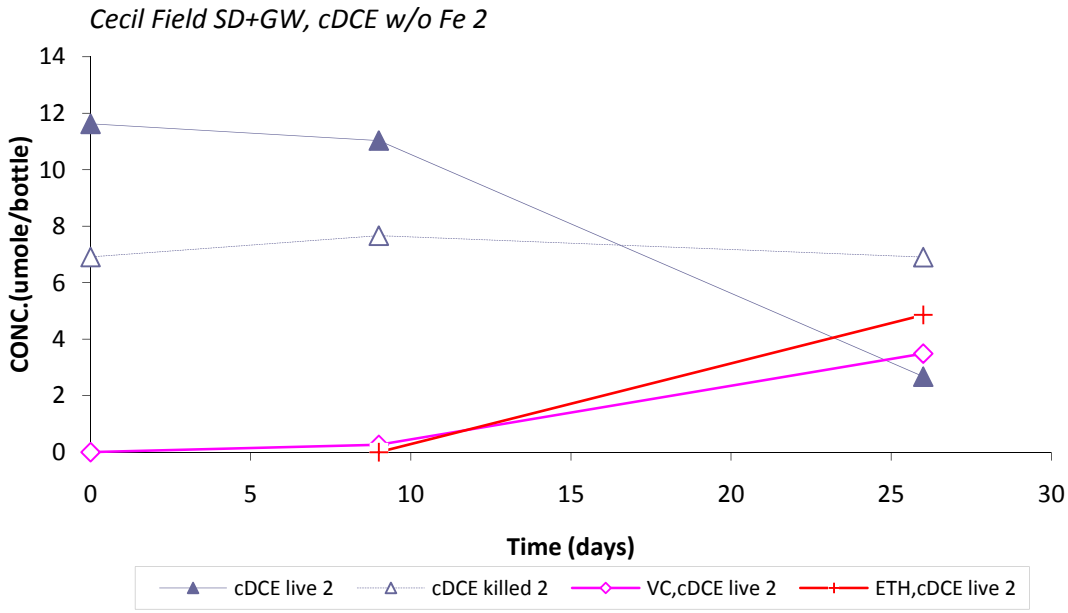


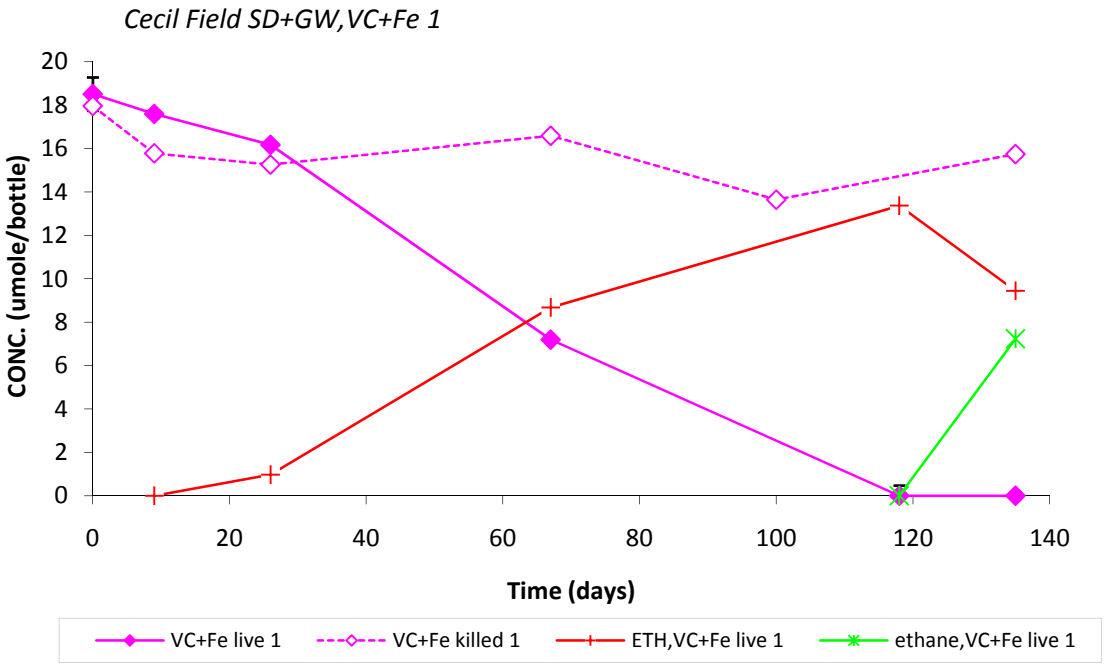
9-A. Site/Source 9: Naval Air Station, Cecil Field, Sediment+Groundwater:

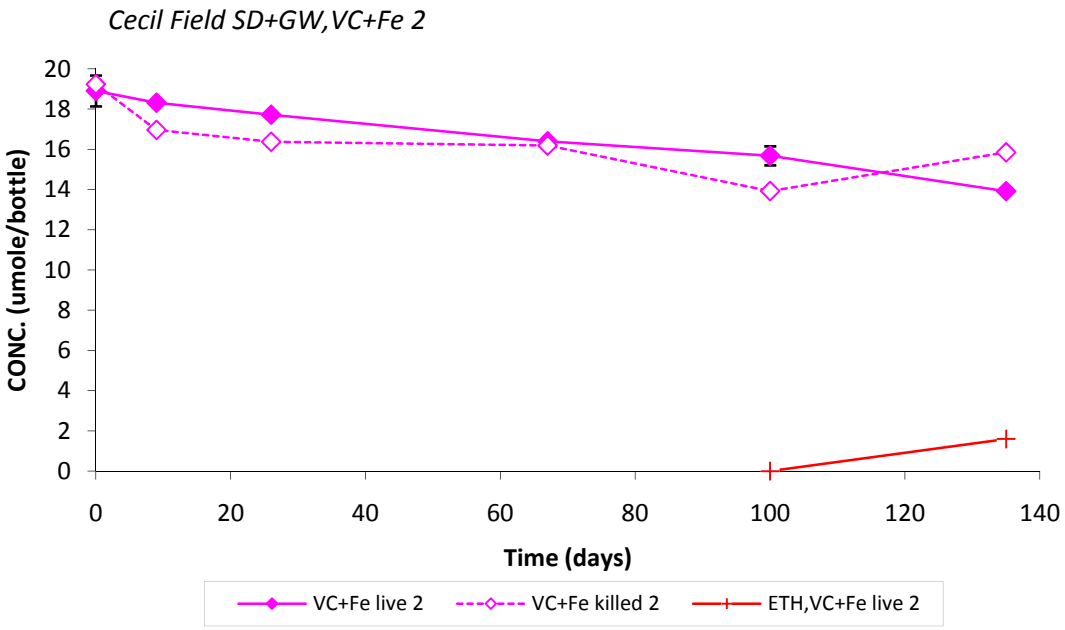


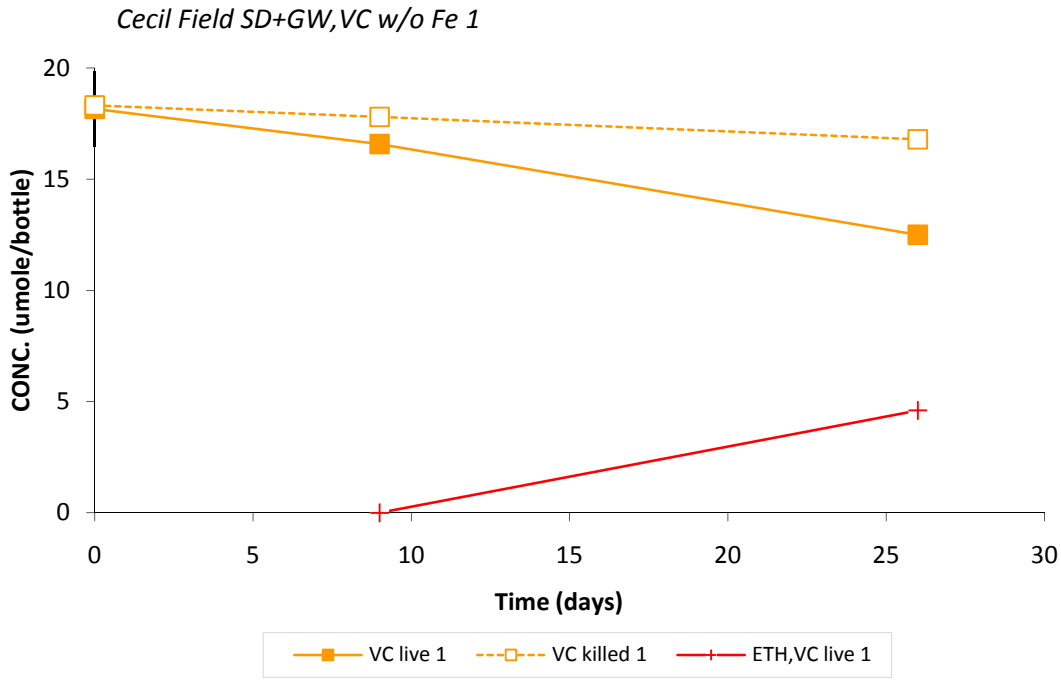

Cecil Field SD+GW, cDCE+Fe 2
CONC.(umole/bottle)
Time (days)
cDCE+Fe live 2
cDCE+Fe killed 2
VC,cDCE+Fe live 2
ETH,cDCE+Fe live 2

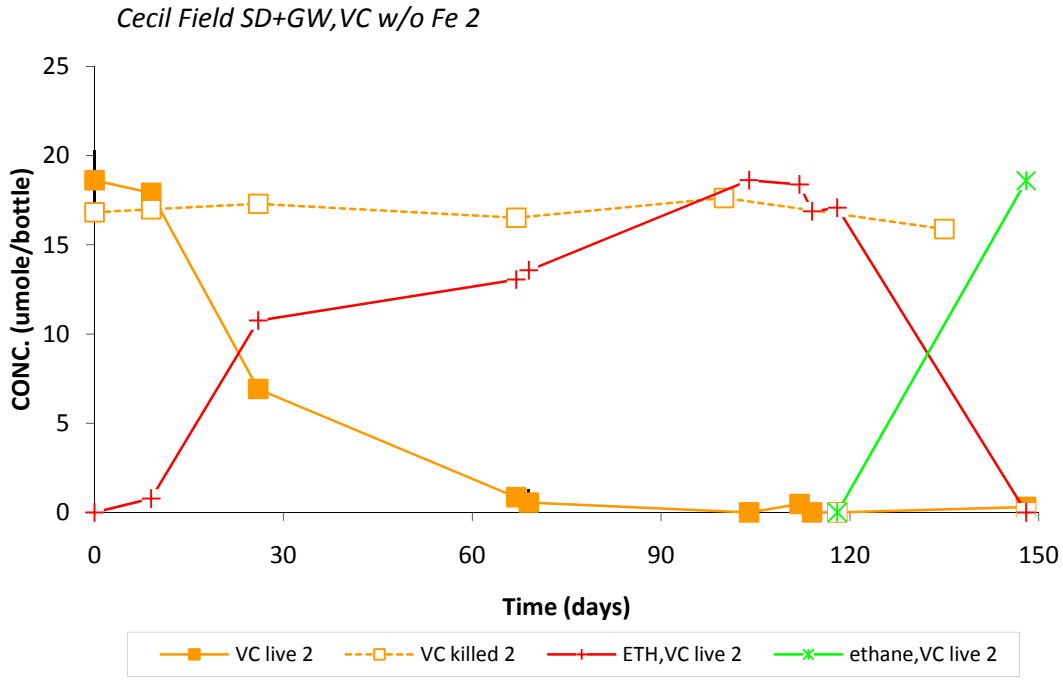

Cecil Field SD+GW, cDCE w/o Fe 1
CONC.(umole/bottle)
Time (days)
cDCE live 1
cDCE killed 1
VC,cDCE live 1
ETH,cDCE live 1
ethane,cDCE live 1

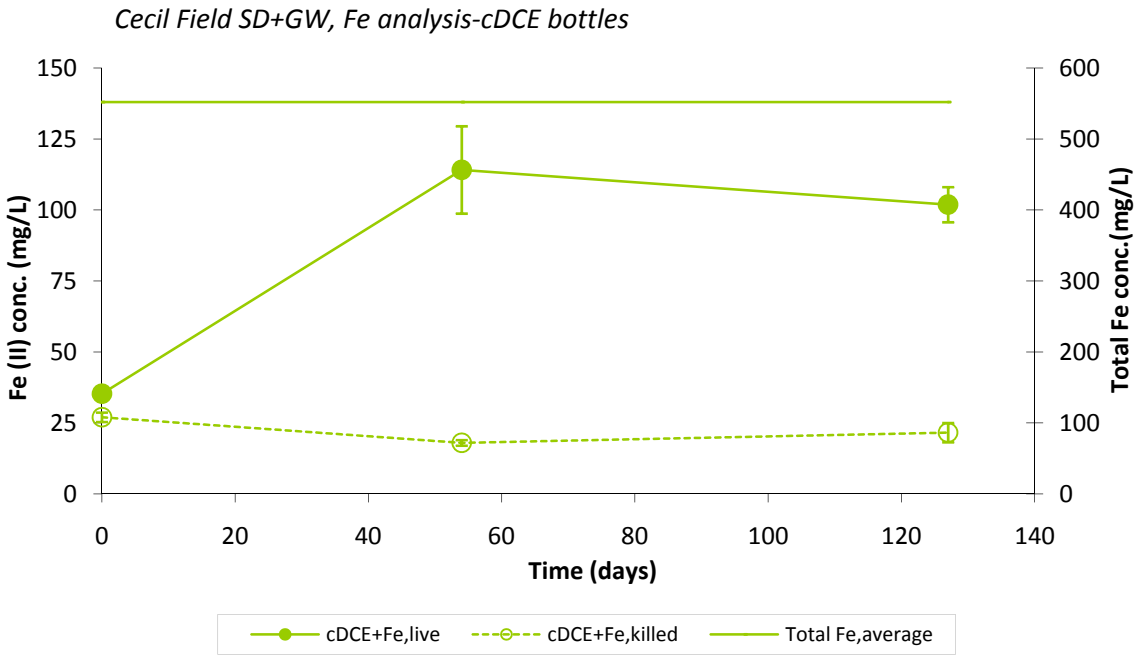

Cecil Field SD+GW, cDCE w/o Fe 2
CONC.(umole/bottle)
Time (days)
cDCE live 2
cDCE killed 2
VC,cDCE live 2
ETH,cDCE live 2

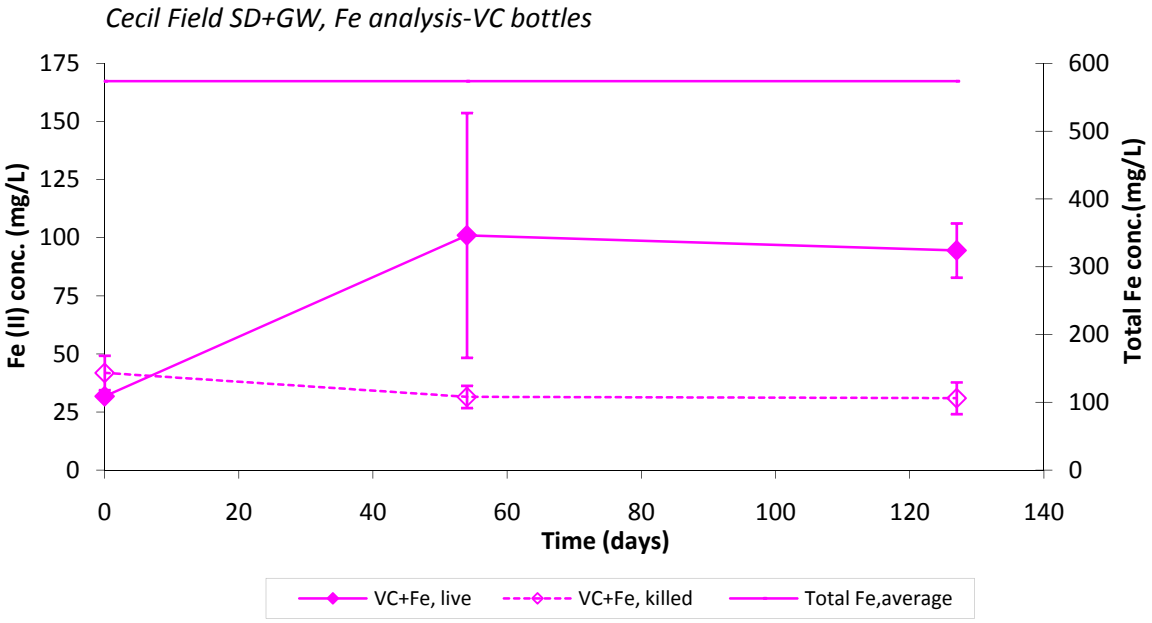

Cecil Field SD+GW,VC+Fe 1
CONC. (umole/bottle)
Time (days)
VC+Fe live 1
VC+Fe killed 1
ETH,VC+Fe live 1
ethane,VC+Fe live 1


Cecil Field SD+GW,VC+Fe 2
CONC. (umole/bottle)
Time (days)
VC+Fe live 2
VC+Fe killed 2
ETH,VC+Fe live 2

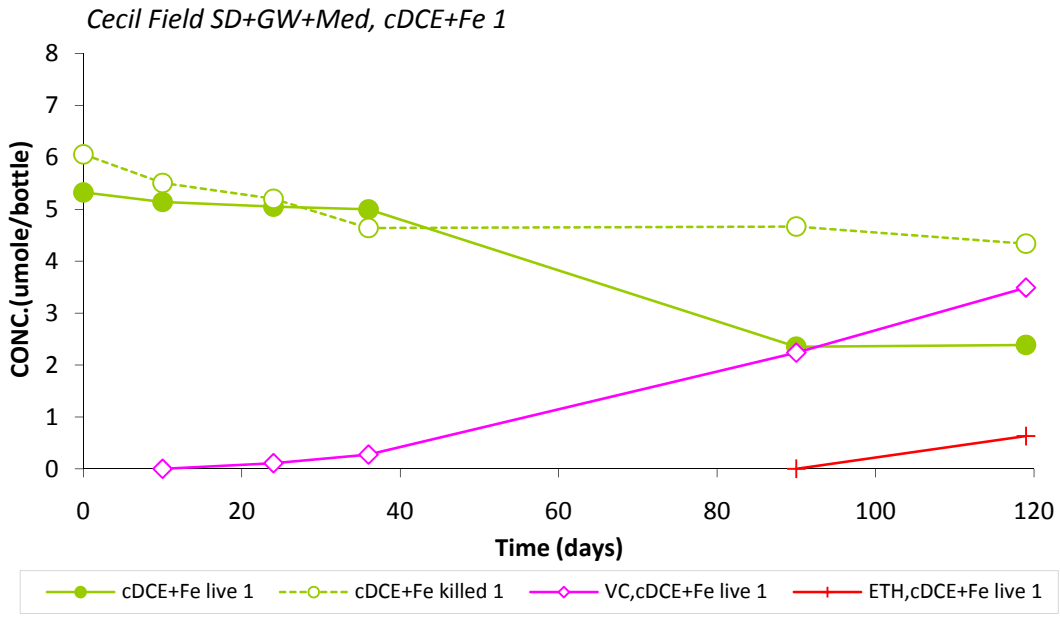

Cecil Field SD+GW,VC w/o Fe 1
CONC. (umole/bottle)
Time (days)
VC live 1
VC killed 1
ETH,VC live 1

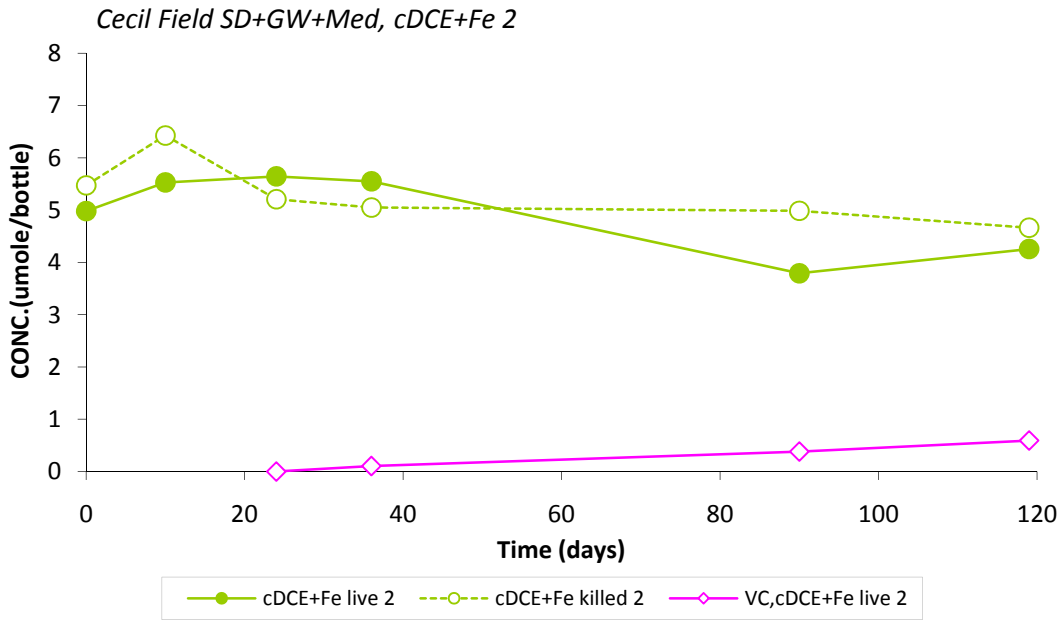

Cecil Field SD+GW,VC w/o Fe 2
CONC. (umole/bottle)
25
20
15
10
5
0
0
30
60
90
120
150
Time (days)
VC live 2
VC killed 2
ETH,VC live 2
ethane,VC live 2

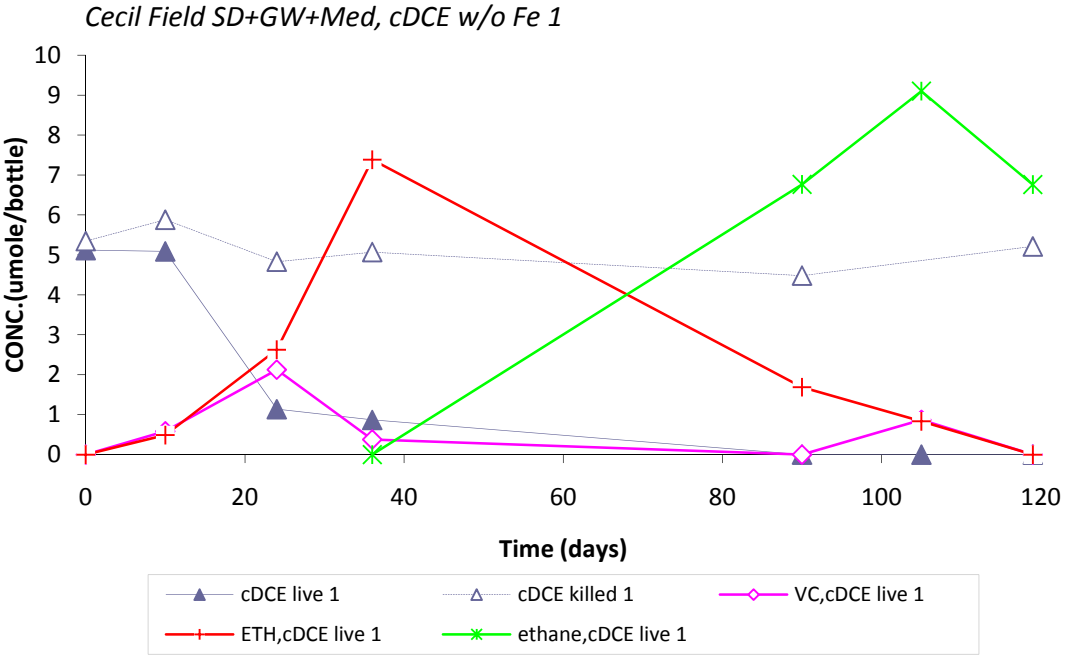

Cecil Field SD+GW, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
150
125
100
75
50
25
0
Total Fe conc.(mg/L)
600
500
400
300
200
100
0
0
20
40
60
80
100
120
140
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

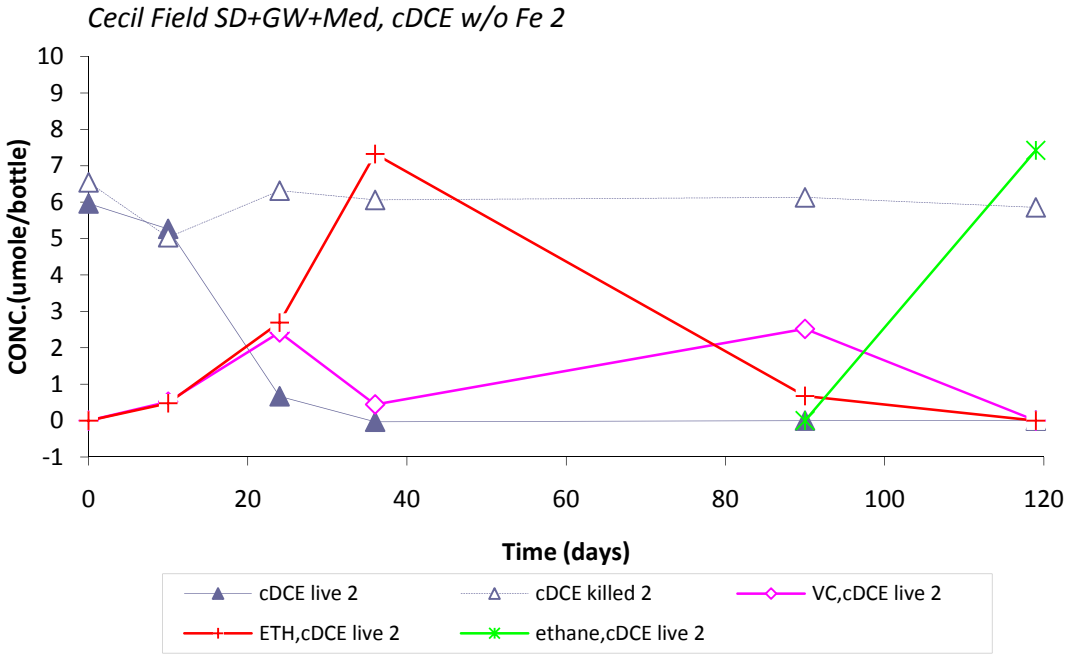


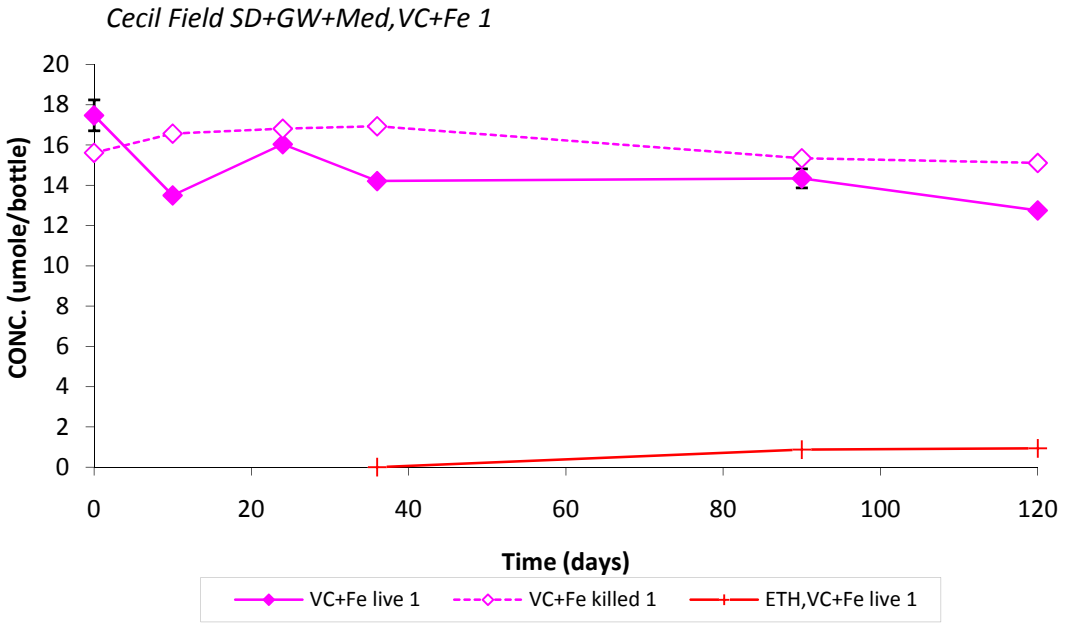
9-B. Site/Source 9: NAS Cecil Field, Sediment+Groundwater+Medium:

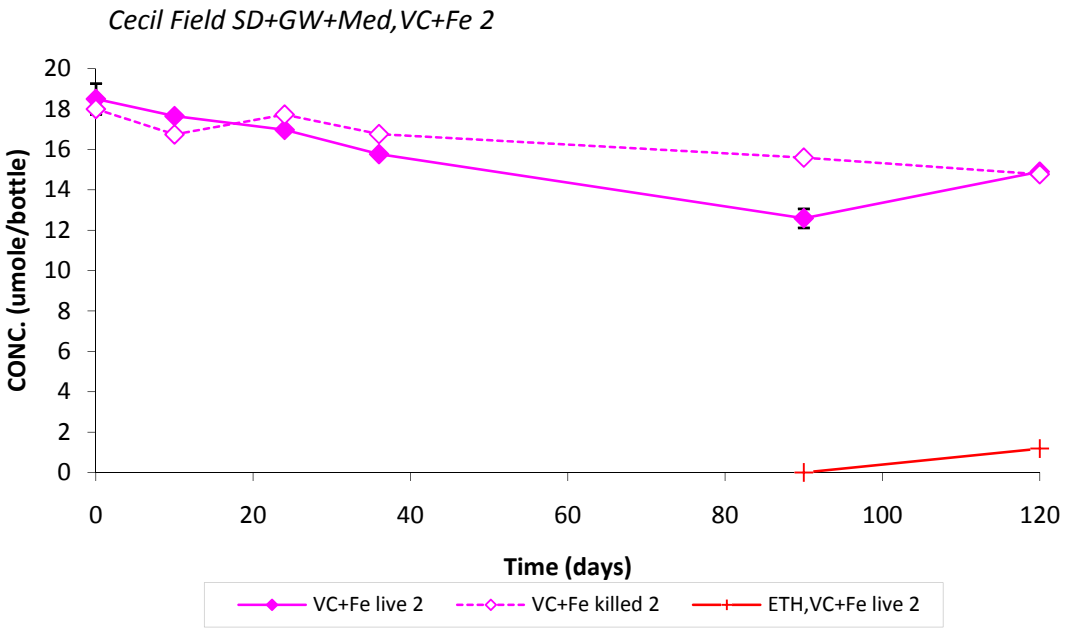


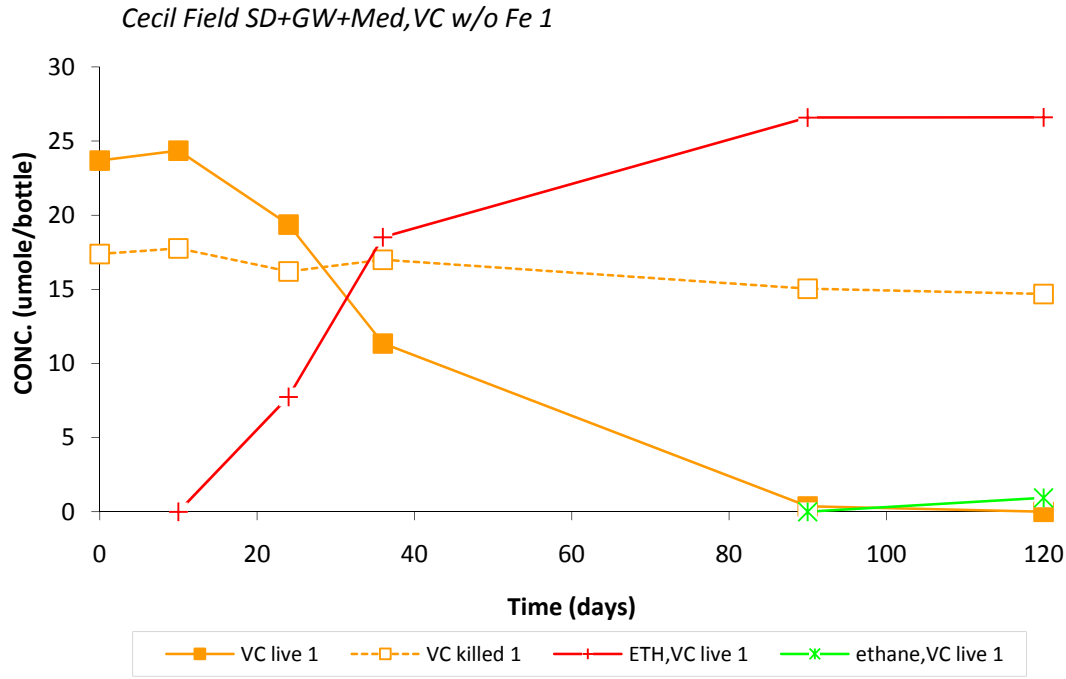

Cecil Field SD+GW+Med, cDCE+Fe 2
CONC.(umole/bottle)
Time (days)
cDCE+Fe live 2
cDCE+Fe killed 2
VC,cDCE+Fe live 2

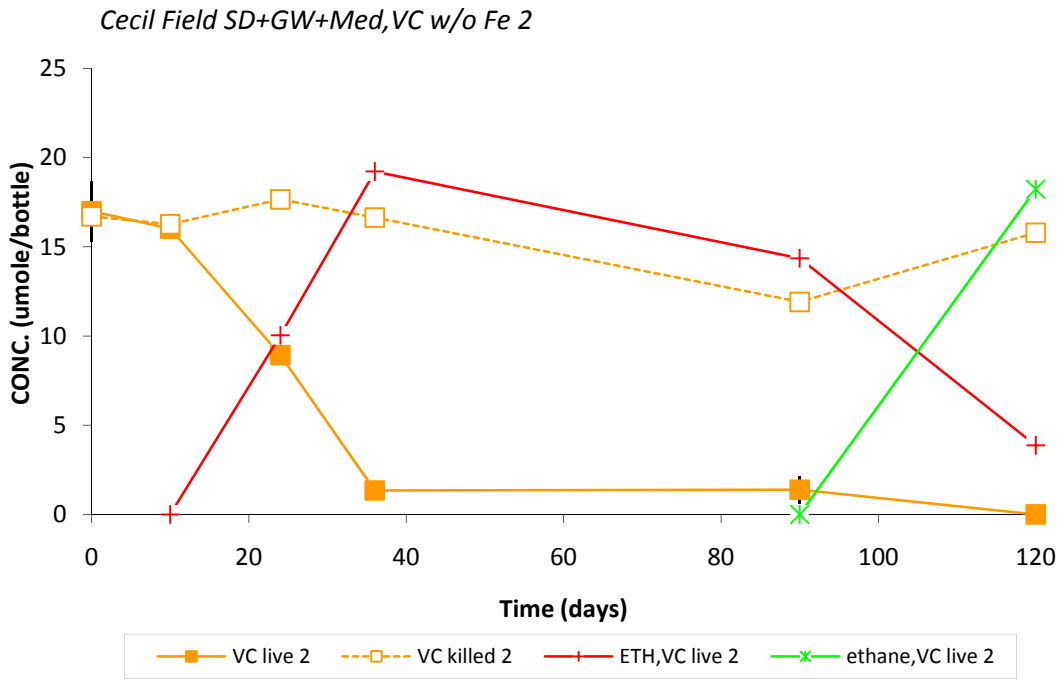

Cecil Field SD+GW+Med, cDCE w/o Fe 1
CONC.(umole/bottle)
Time (days)
cDCE live 1
cDCE killed 1
VC,cDCE live 1
ETH,cDCE live 1
ethane,cDCE live 1

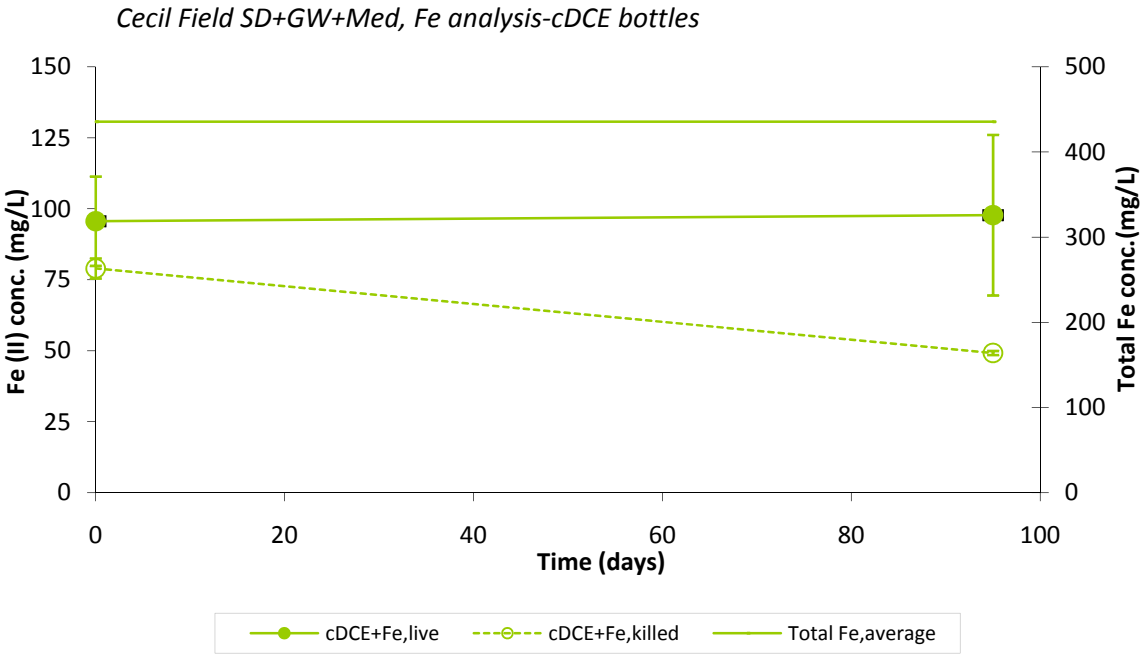

Cecil Field SD+GW+Med, cDCE w/o Fe 2
CONC.(umole/bottle)
10
9
8
7
6
5
4
3
2
1
0
-1
0
20
40
60
80
100
120
Time (days)
cDCE live 2
cDCE killed 2
VC,cDCE live 2
ETH,cDCE live 2
ethane,cDCE live 2

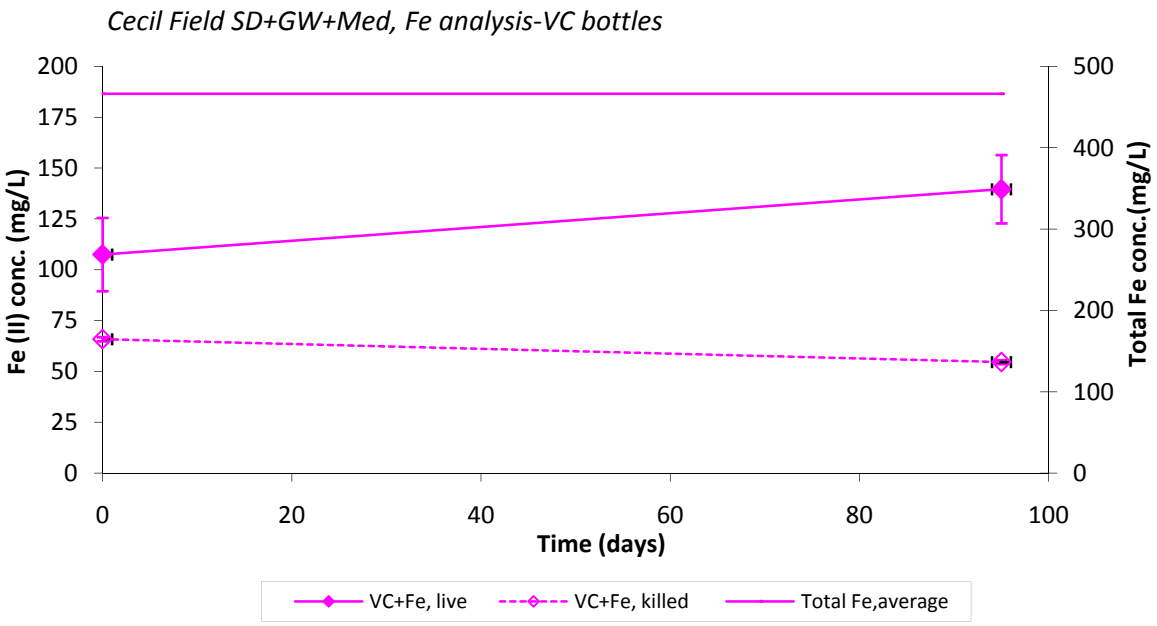

Cecil Field SD+GW+Med,VC+Fe 1
CONC. (umole/bottle)
20
18
16
14
12
10
8
6
4
2
0
0
20
40
60
80
100
120
Time (days)
VC+Fe live 1
VC+Fe killed 1
ETH,VC+Fe live 1


Cecil Field SD+GW+Med,VC+Fe 2
CONC. (umole/bottle)
Time (days)
VC+Fe live 2
VC+Fe killed 2
ETH,VC+Fe live 2

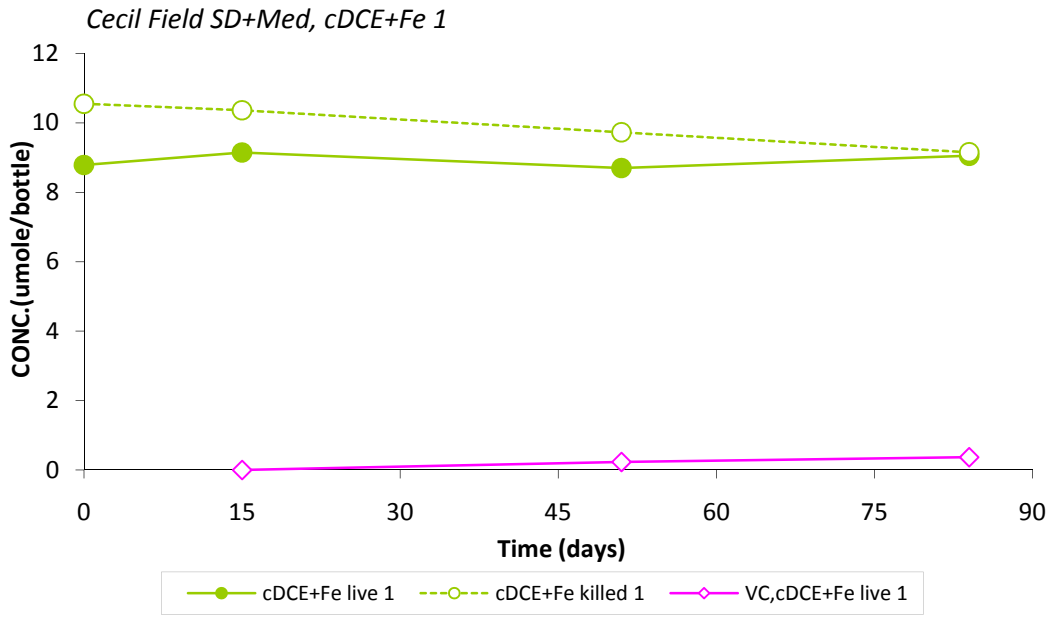

Cecil Field SD+GW+Med,VC w/o Fe 1
CONC. (umole/bottle)
Time (days)
VC live 1
VC killed 1
ETH,VC live 1
ethane,VC live 1

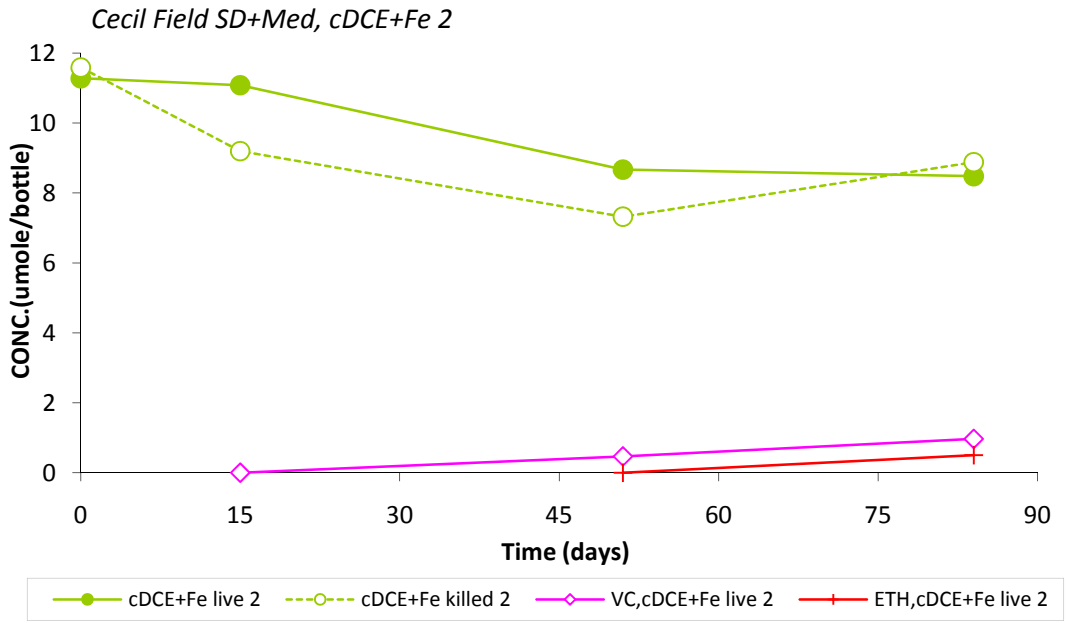

Cecil Field SD+GW+Med,VC w/o Fe 2
CONC. (umole/bottle)
25
20
15
10
5
0
0
20
40
60
80
100
120
Time (days)
VC live 2
VC killed 2
ETH,VC live 2
ethane,VC live 2

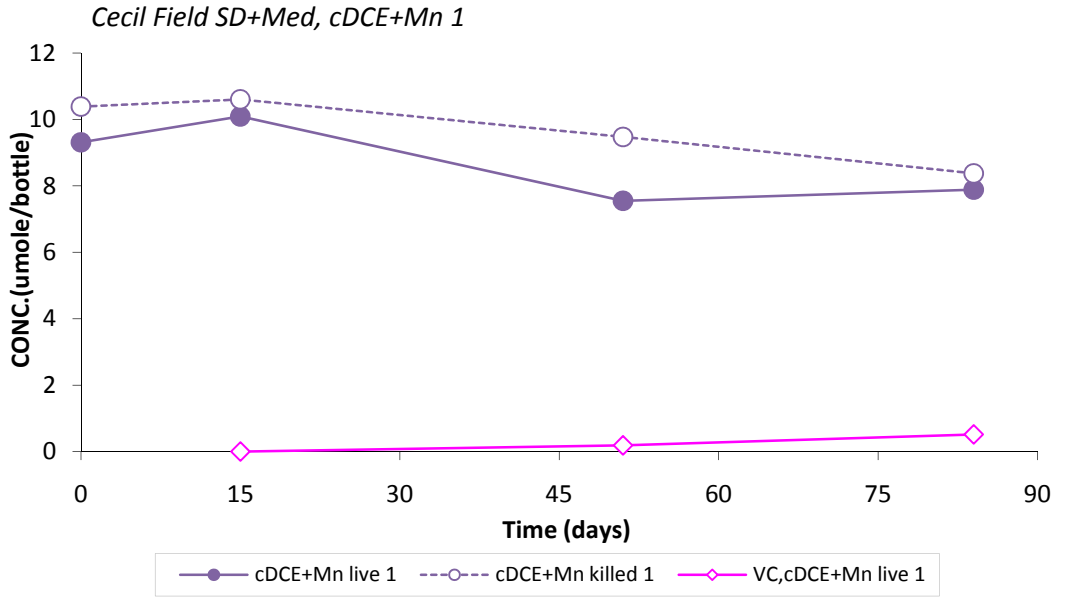

Cecil Field SD+GW+Med, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
150
125
100
75
50
25
0
Total Fe conc.(mg/L)
500
400
300
200
100
0
0
20
40
60
80
100
Time (days)
cDCE+Fe,live
cDCE+Fe,killed
Total Fe,average

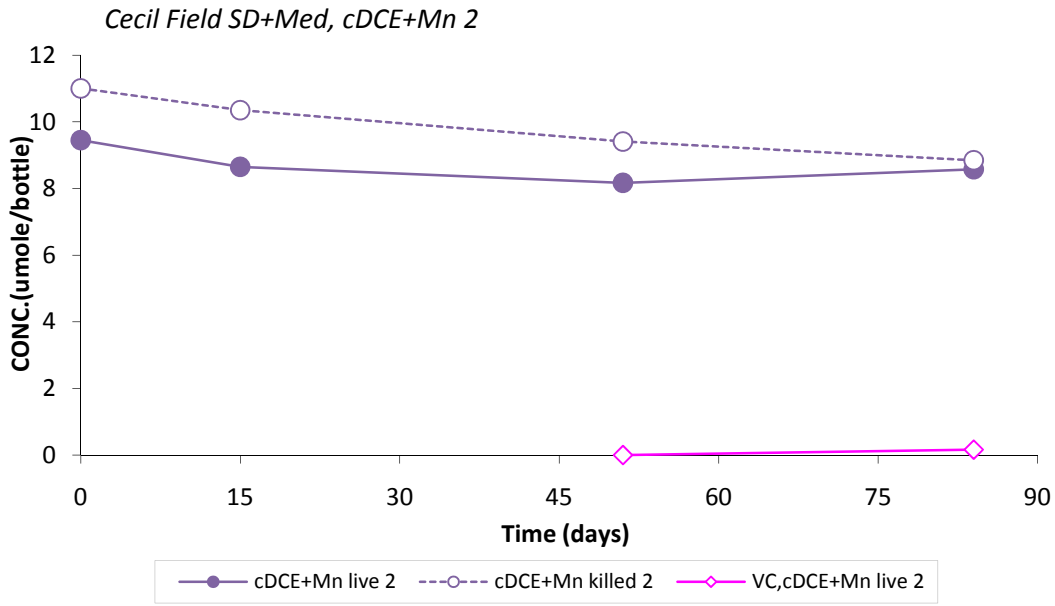


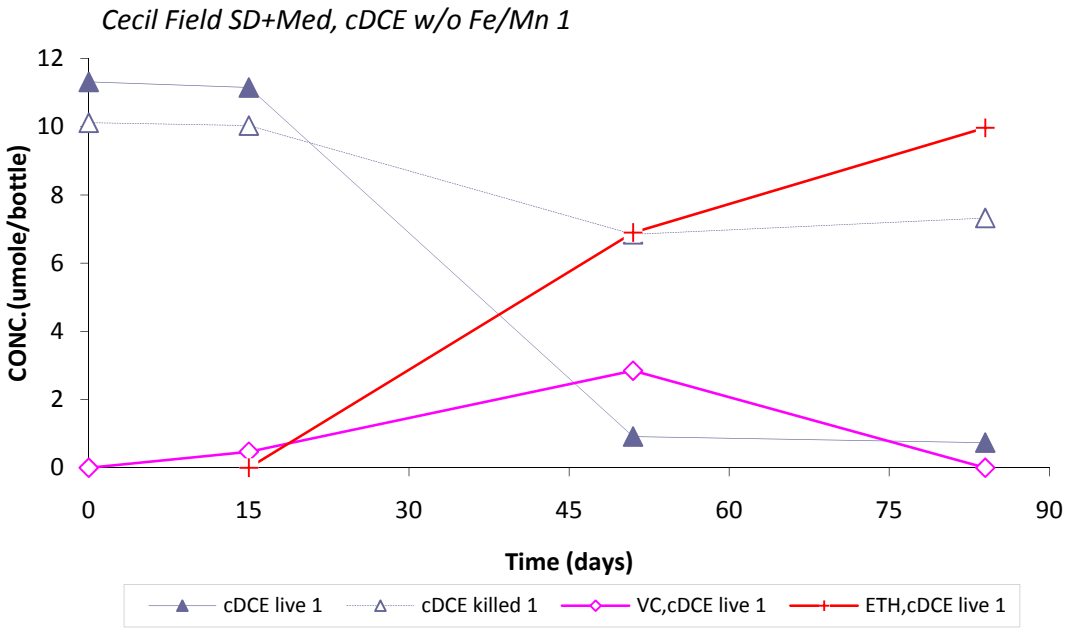
9-C. Site/Source 9: NAS Cecil Field, Sediment+Medium:

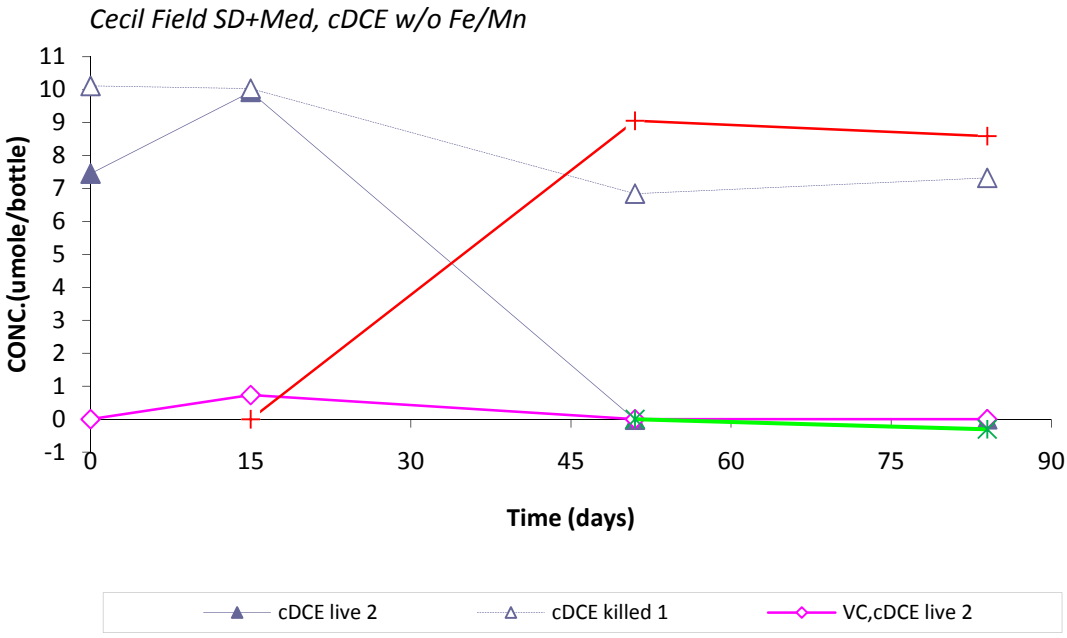


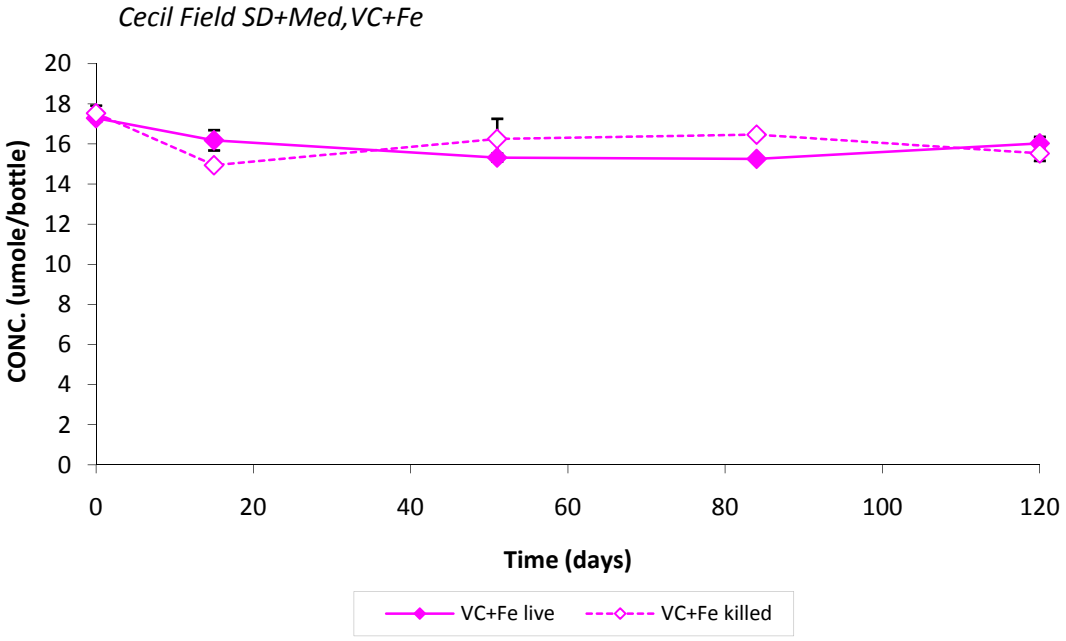

Cecil Field SD+Med, cDCE+Fe 2
CONC.(umole/bottle)
Time (days)
0
2
4
6
8
10
12
0
15
30
45
60
75
90
cDCE+Fe live 2
cDCE+Fe killed 2
VC,cDCE+Fe live 2
ETH,cDCE+Fe live 2

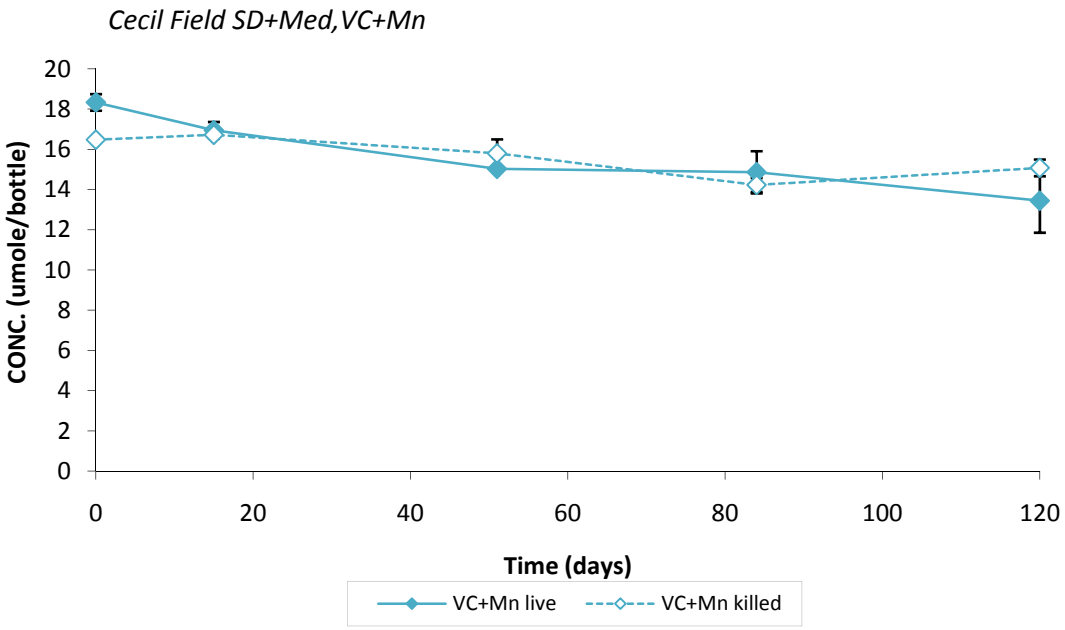

Cecil Field SD+Med, cDCE+Mn 1
CONC.(umole/bottle)
Time (days)
0
2
4
6
8
10
12
0
15
30
45
60
75
90
cDCE+Mn live 1
cDCE+Mn killed 1
VC,cDCE+Mn live 1

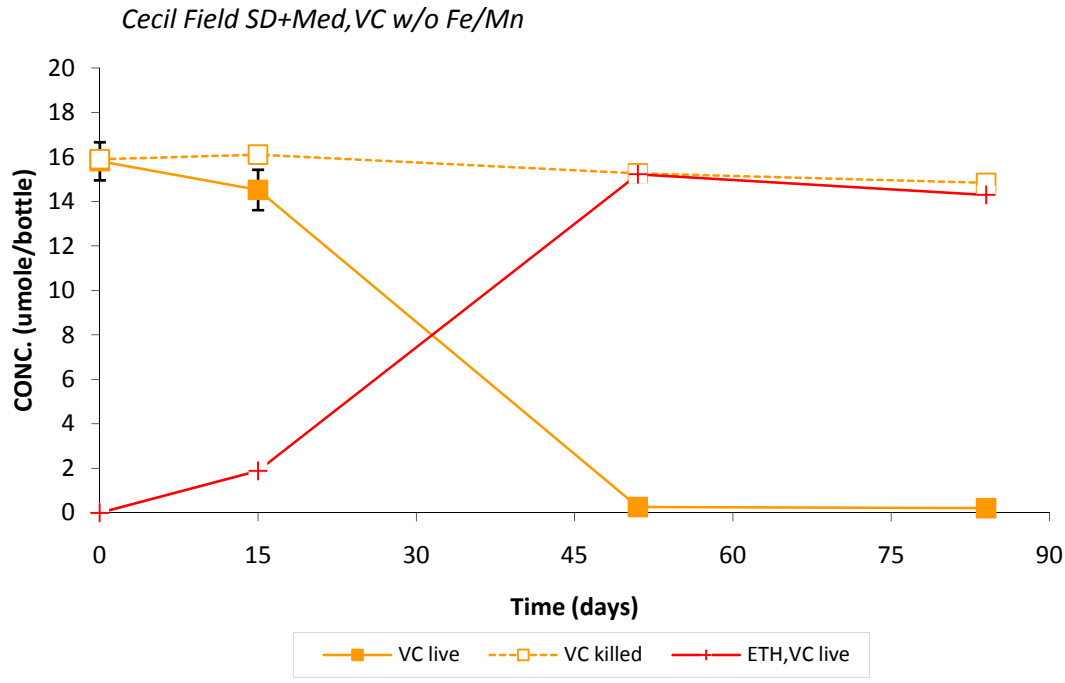

Cecil Field SD+Med, cDCE+Mn 2
CONC.(umole/bottle)
12
10
8
6
4
2
0
0
15
30
45
60
75
90
Time (days)
cDCE+Mn live 2
cDCE+Mn killed 2
VC,cDCE+Mn live 2

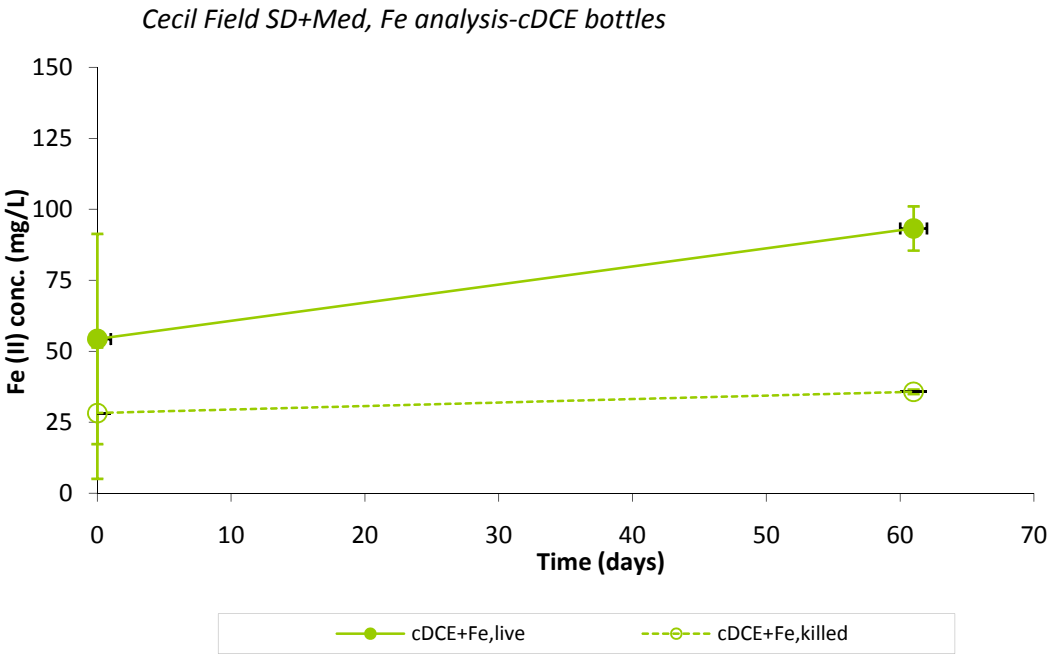

Cecil Field SD+Med, cDCE w/o Fe/Mn 1
CONC.(umole/bottle)
12
10
8
6
4
2
0
0
15
30
45
60
75
90
Time (days)
cDCE live 1
cDCE killed 1
VC,cDCE live 1
ETH,cDCE live 1

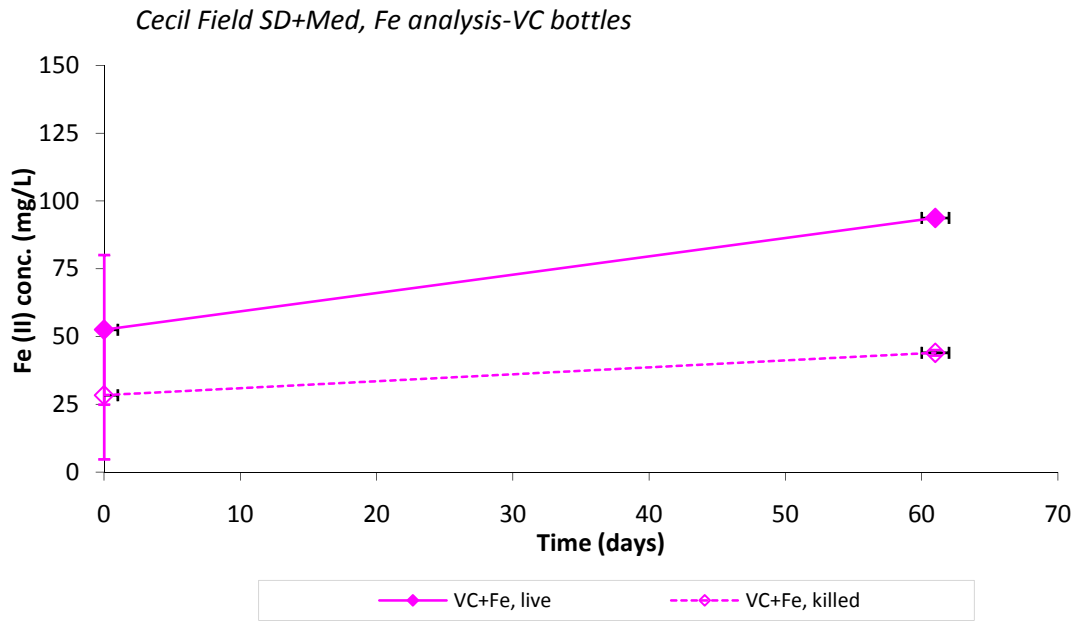

Cecil Field SD+Med, cDCE w/o Fe/Mn
CONC.(umole/bottle)
Time (days)
cDCE live 2
cDCE killed 1
VC,cDCE live 2


Cecil Field SD+Med,VC+Fe
CONC. (umole/bottle)
Time (days)
VC+Fe live
VC+Fe killed

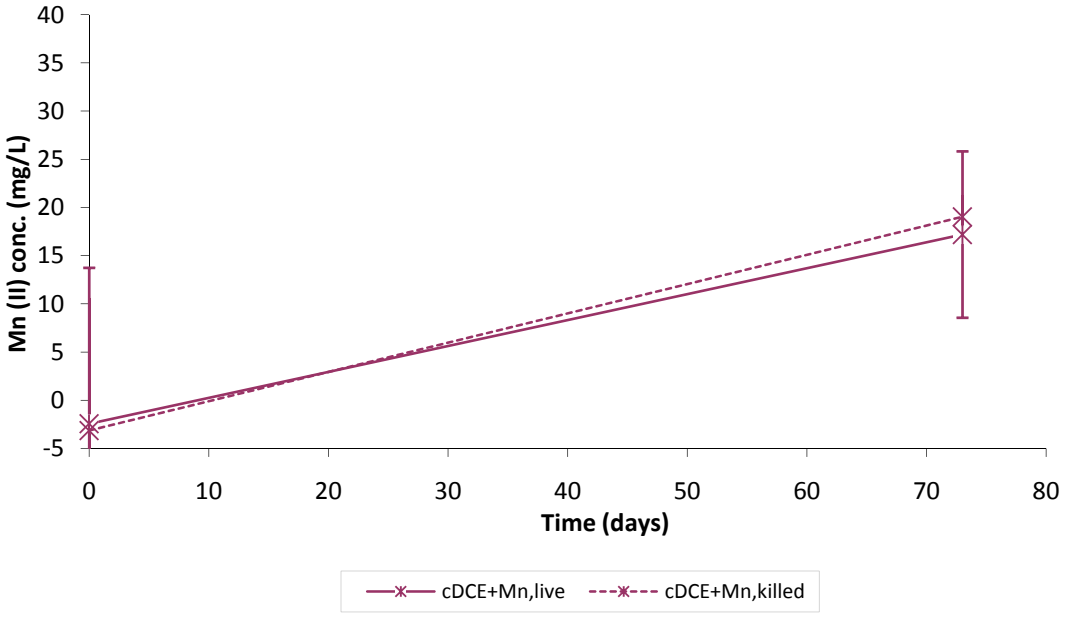

Cecil Field SD+Med,VC+Mn
CONC. (umole/bottle)
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100
120
Time (days)
VC+Mn live
VC+Mn killed


Cecil Field SD+Med,VC w/o Fe/Mn
CONC. (umole/bottle)
0
2
4
6
8
10
12
14
16
18
20
0
15
30
45
60
75
90
Time (days)
VC live
VC killed
ETH,VC live

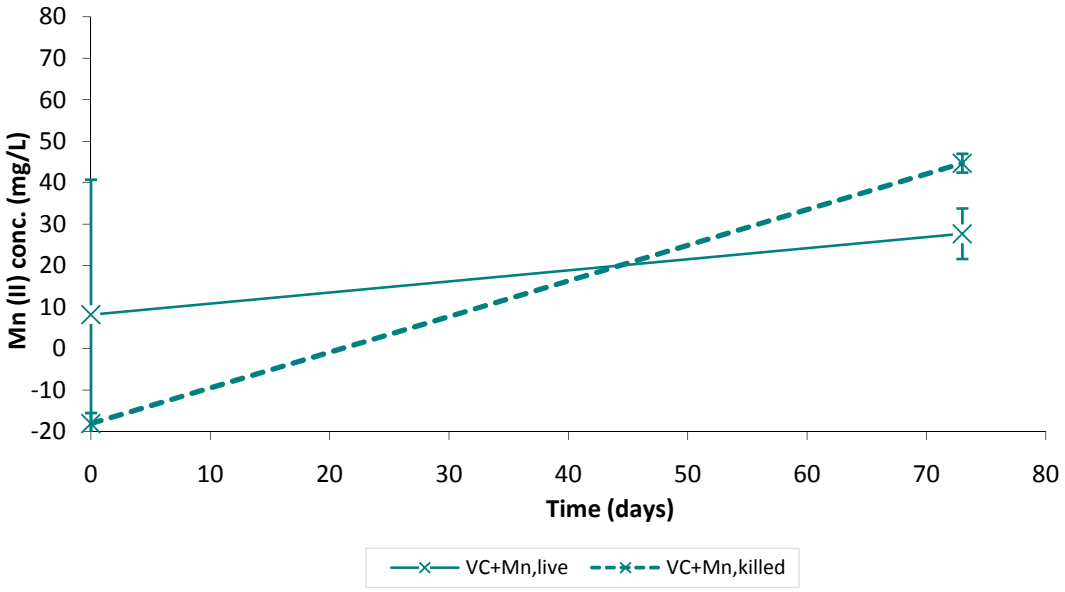

Cecil Field SD+Med, Fe analysis-cDCE bottles
Fe (II) conc. (mg/L)
150
125
100
75
50
25
0
0
10
20
30
40
50
60
70
Time (days)
cDCE+Fe,live
cDCE+Fe,killed


Cecil Field SD+Med, Fe analysis-VC bottles
Fe (II) conc. (mg/L)
150
125
100
75
50
25
0
0
10
20
30
40
50
60
70
Time (days)
VC+Fe, live
VC+Fe, killed

Cecil Field SD+Med, Mn analysis-cDCE bottles



Cecil Field SD+Med, Mn analysis-VC bottles

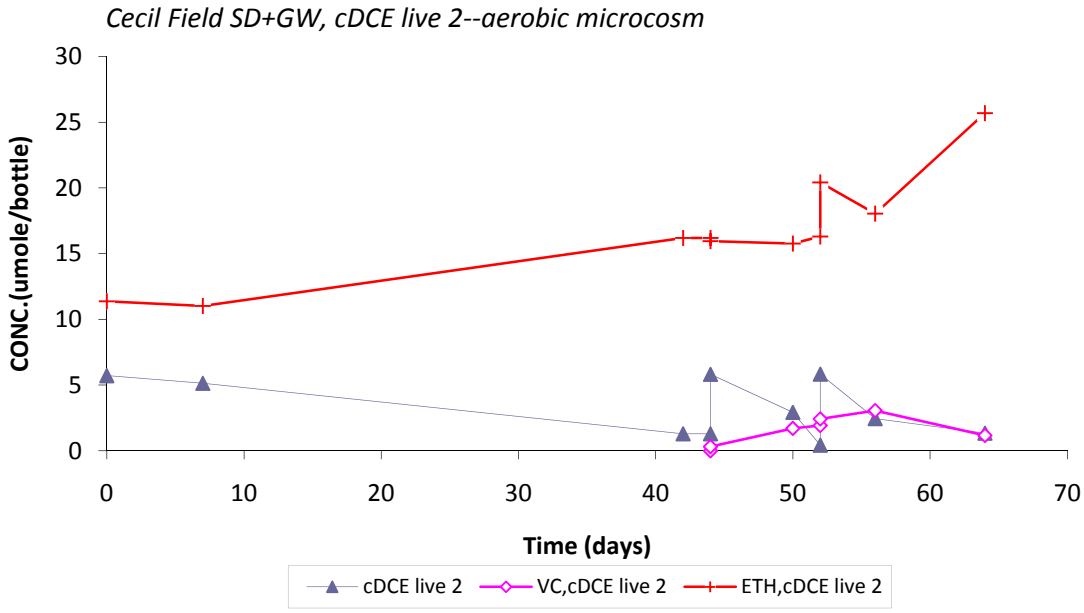


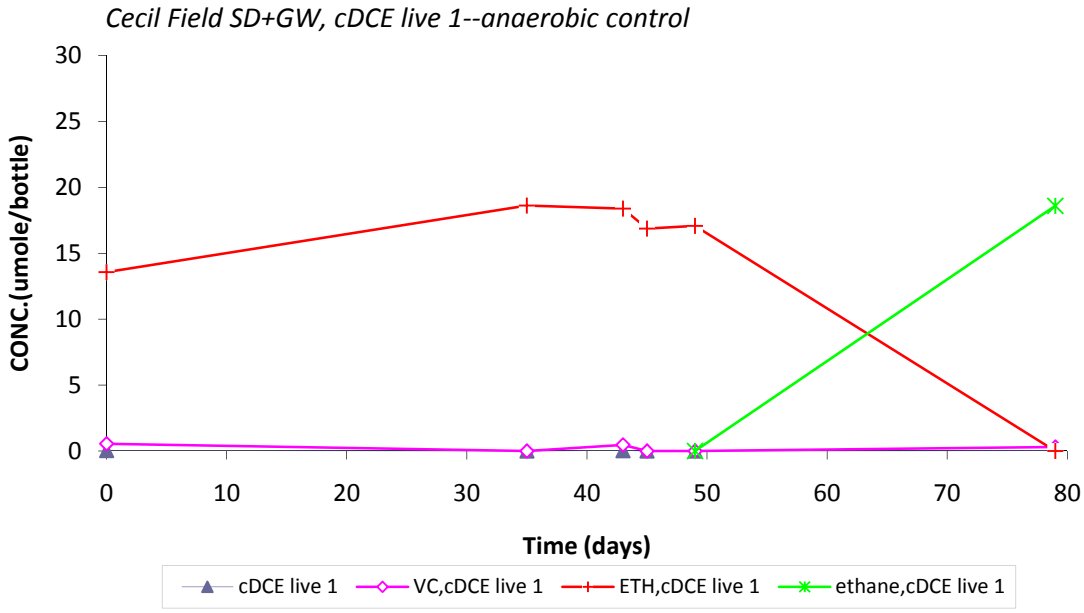
APPENDIX IV: RESULTS FOR AEROBIC MICROCOSMS

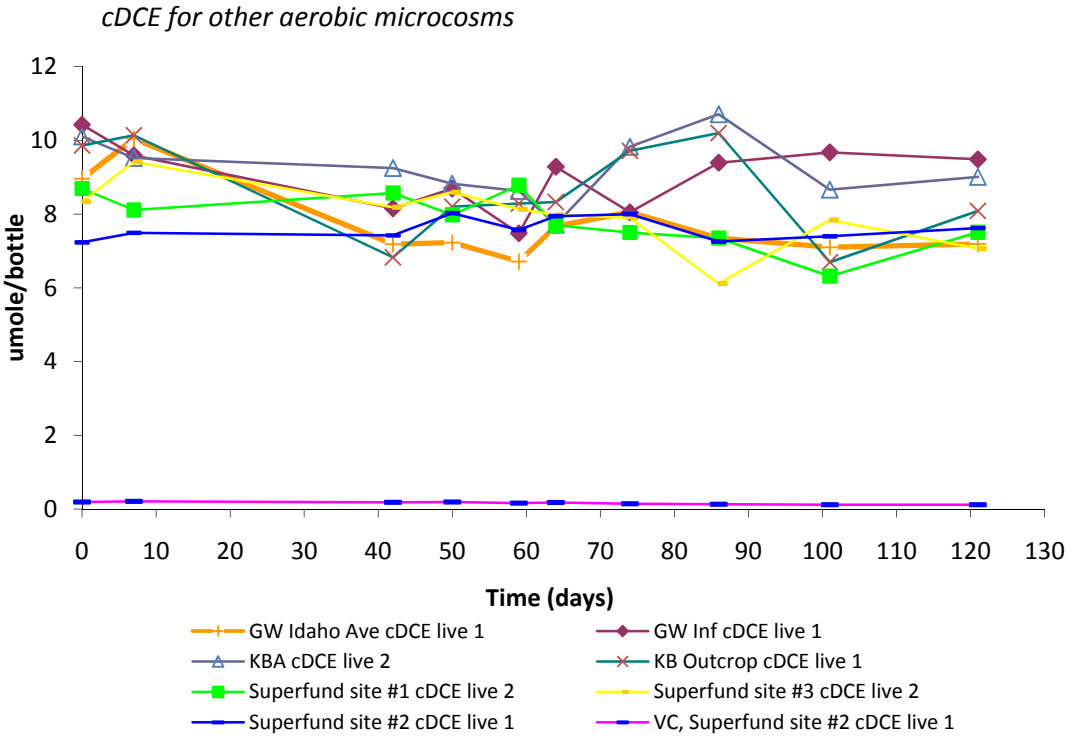
Table IV. Microcosms for Aerobic Experiment.

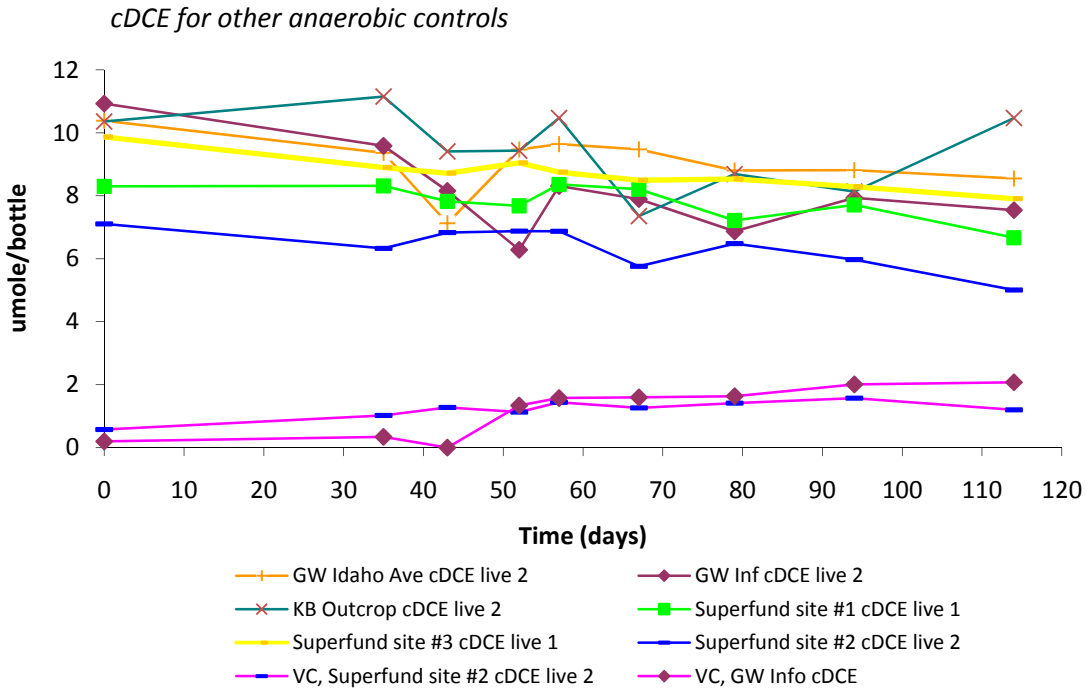
No.	Site	Experiment	Control
1	Plattsburgh AFB, Influent to the groundwater treatment plant	cDCE live 1	cDCE live 2
		VC+Fe live 1	VC+Fe live 2
2	Plattsburgh AFB, Idaho Avenue discharge pipe	cDCE live 2	cDCE live 1
		VC live 2	VC live 1
3	NSB, Kings Bay KBA-11-13A	cDCE live 2	Broken
		VC live 2	VC live 1
4	NSB, Kings Bay Outcrop	cDCE live 1	cDCE live 2
		VC live 2	VC live 1
5	Unidentified Superfund Site, Subset #1	cDCE live 2	cDCE live 1
		VC live 2	VC live 1
6	Unidentified Superfund Site, Subset #2	cDCE live 2	cDCE live 1
		VC live 2	Broken
7	Unidentified Superfund Site, Subset #3-(a)	cDCE live 2	cDCE live 1
		VC live 2	VC live 1
8	NAS Cecil Field, Sediment+Groundwater	cDCE live 2	cDCE live 1
		VC live 1	VC live 2
Total		16	14

1-A. cDCE-fed Aerobic Microcosms

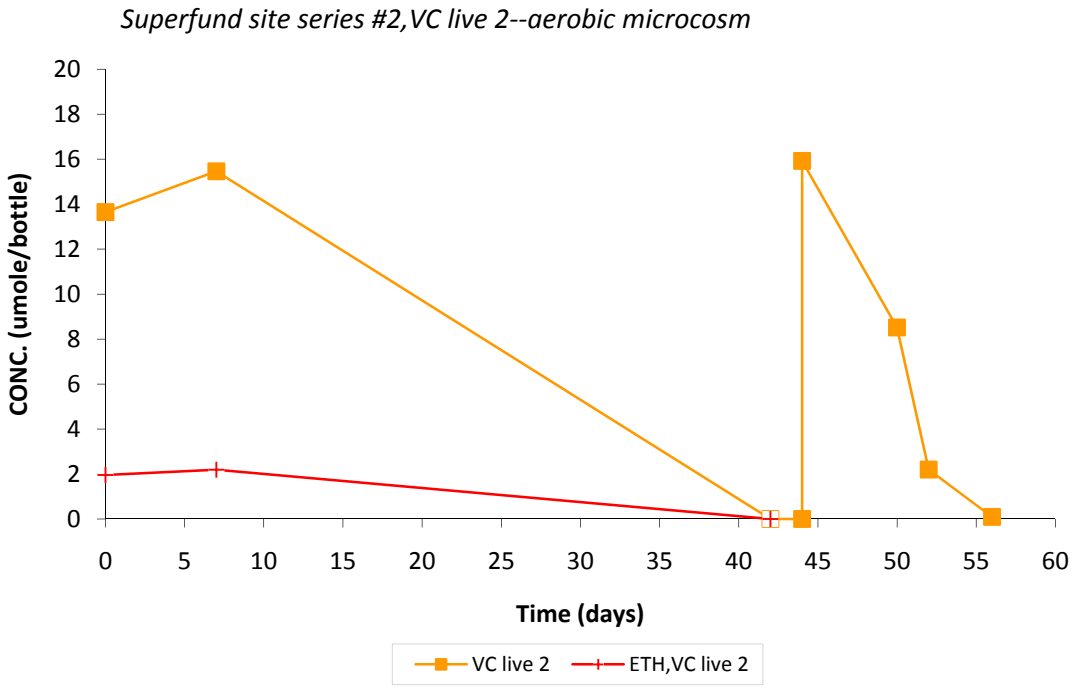


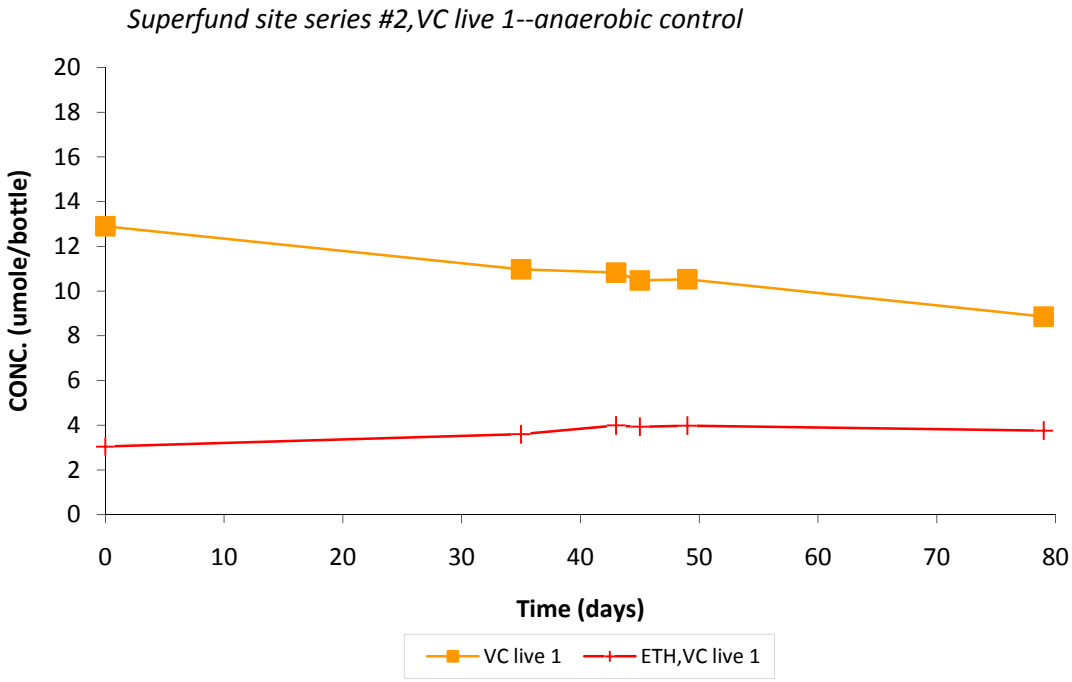



cDCE for other aerobic microcosms
umole/bottle
Time (days)
GW Idaho Ave cDCE live 1
GW Inf cDCE live 1
KBA cDCE live 2
KB Outcrop cDCE live 1
Superfund site #1 cDCE live 2
Superfund site #3 cDCE live 2
Superfund site #2 cDCE live 1
VC, Superfund site #2 cDCE live 1

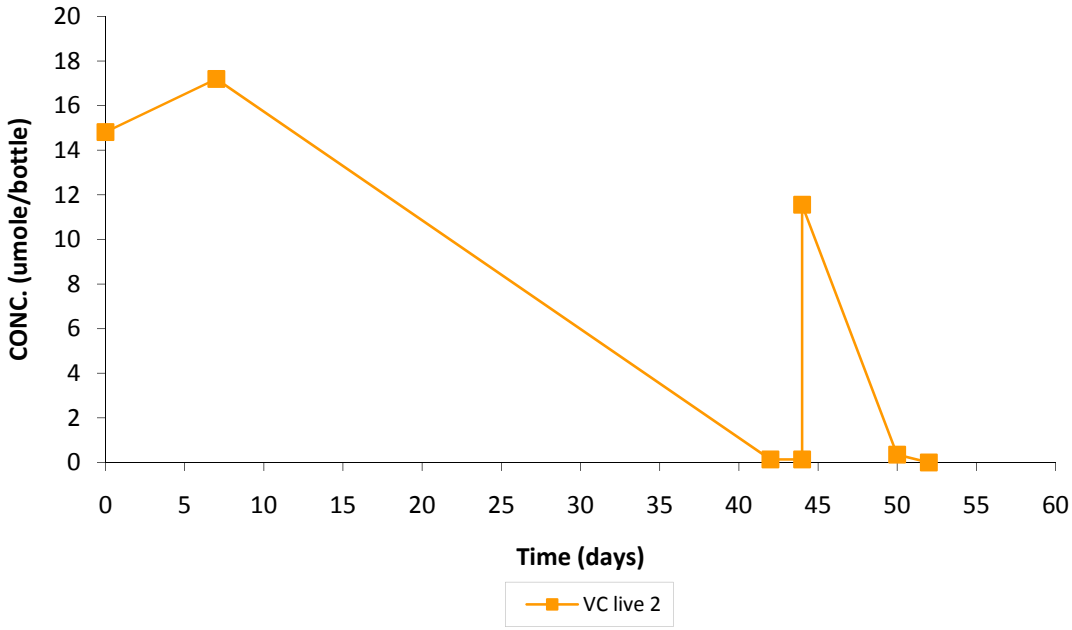

cDCE for other anaerobic controls
umole/bottle
Time (days)
GW Idaho Ave cDCE live 2
GW Inf cDCE live 2
KB Outcrop cDCE live 2
Superfund site #1 cDCE live 1
Superfund site #3 cDCE live 1
Superfund site #2 cDCE live 2
VC, Superfund site #2 cDCE live 2
VC, GW Info cDCE

1-B. VC-fed Aerobic Microcosms

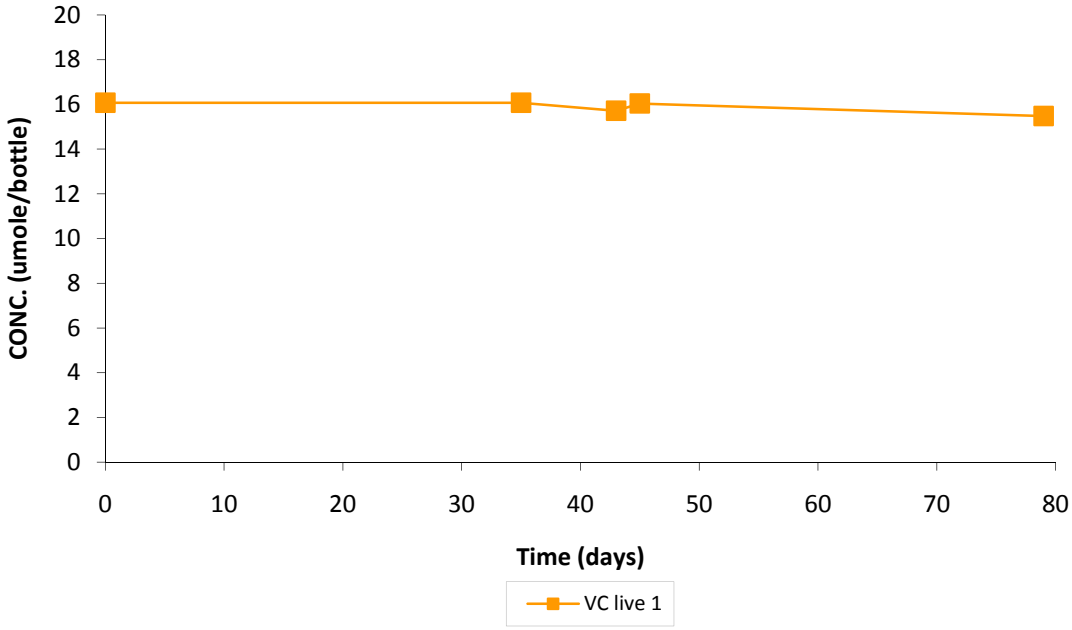


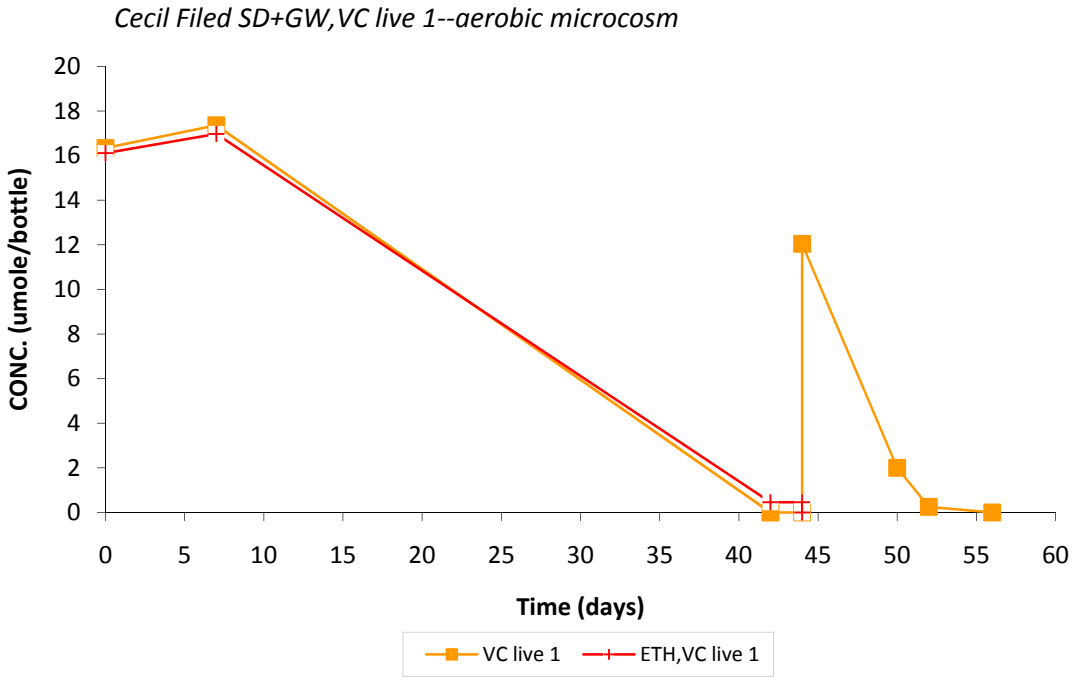


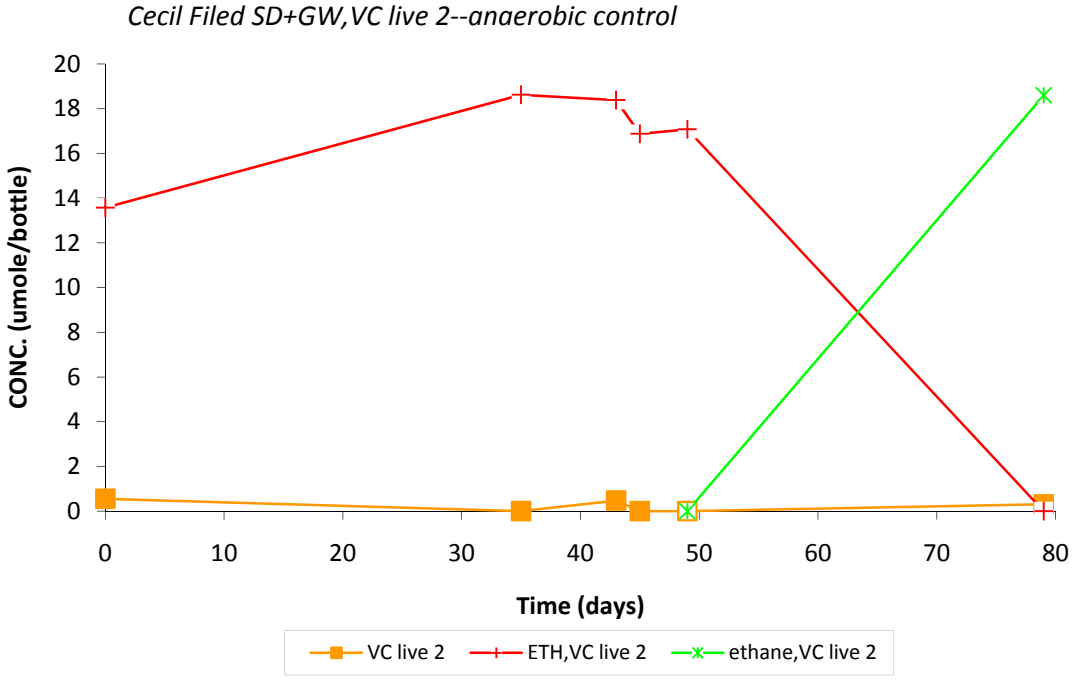
Superfund site series #3-(a),VC live 2--aerobic microcosm

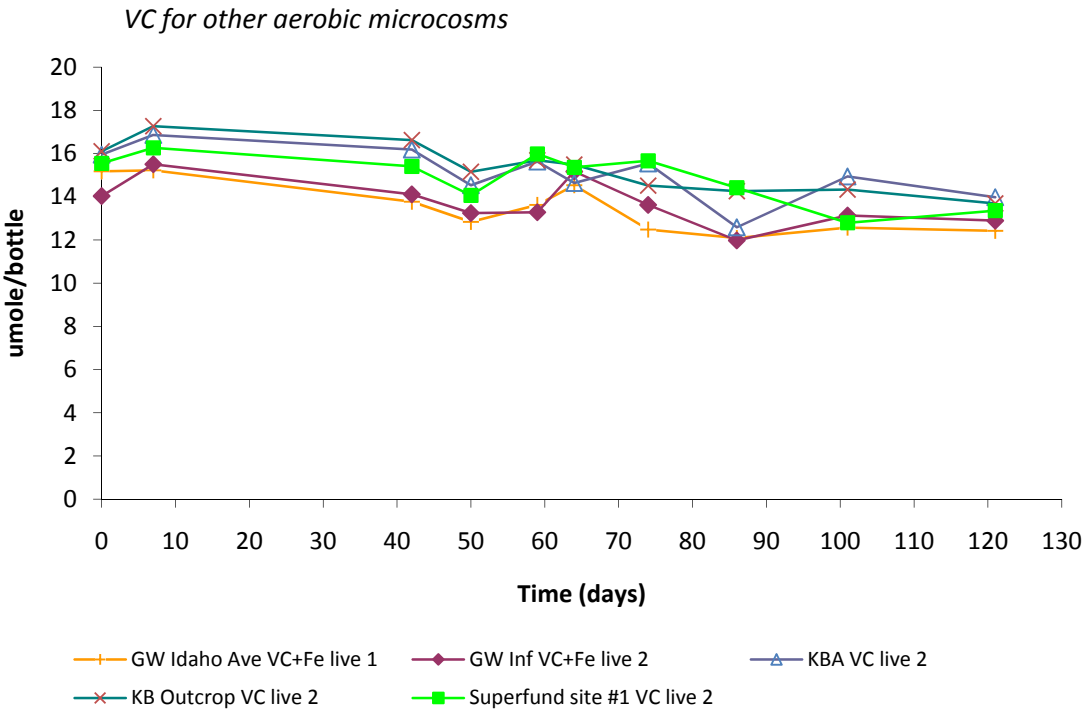


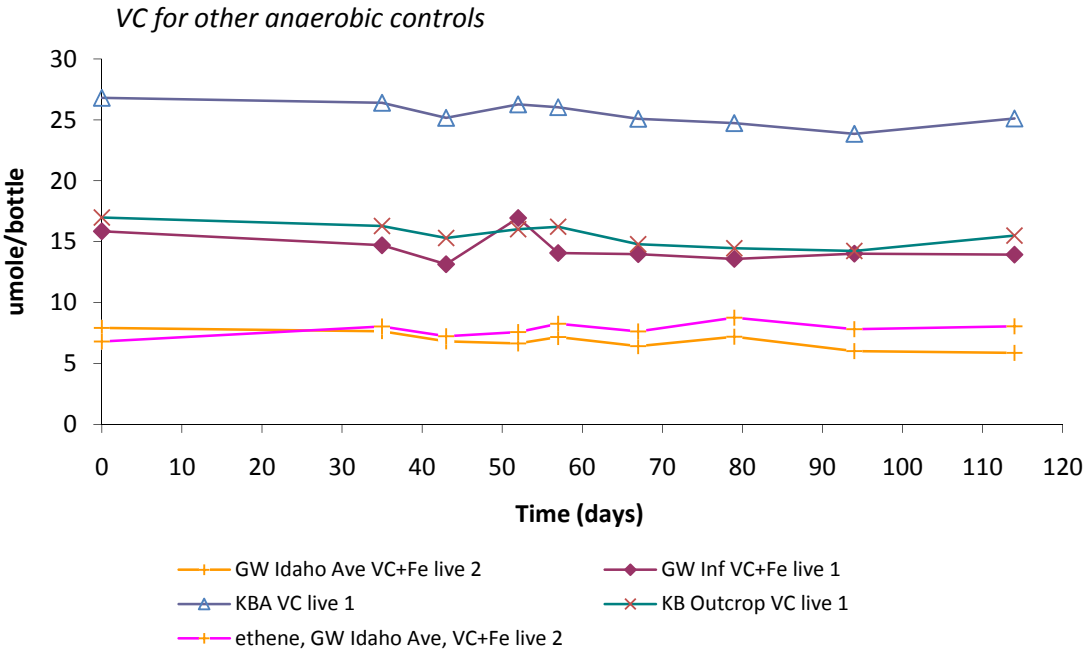
Superfund site series #3-(a),VC live 1--anaerobic control




Cecil Filed SD+GW,VC live 1--aerobic microcosm
CONC. (umole/bottle)
Time (days)
VC live 1
ETH,VC live 1


Cecil Filed SD+GW,VC live 2--anaerobic control
CONC. (umole/bottle)
Time (days)
VC live 2
ETH,VC live 2
ethane,VC live 2


VC for other aerobic microcosms
umole/bottle
Time (days)
GW Idaho Ave VC+Fe live 1
GW Inf VC+Fe live 2
KBA VC live 2
KB Outcrop VC live 2
Superfund site #1 VC live 2


VC for other anaerobic controls
umole/bottle
Time (days)
GW Idaho Ave VC+Fe live 2
GW Inf VC+Fe live 1
KBA VC live 1
KB Outcrop VC live 1
ethene, GW Idaho Ave, VC+Fe live 2

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